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Bachelor Thesis

"Development and Validation of Intracardiac Electrograms Software Tool for Analysis of Atrial Fibrillation Biomarkers"

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Abstract

Atrial Fibrillation (AF) is the most common cardiac arrhythmia and is associated with a drastically increase of mortality risk. Mechanisms underlying the initiation and maintenance of AF are not understood yet and current treatments for AF are not completely efficient. Most effective one, around 50% of effectiveness, is the catheter ablation therapy but its long-term effects are reduced. Target ablation is thought to be a solution if AF focal sources were identified. Associated AF biomarkers to identify such zones are shortened action potential durations (APDs) and slow conduction velocities (CV). Identification of those biomarkers could be made through intracardiac electrogram (EGM) signals, however, they are very complex to interpret and there is still no validated method to perform it.

The aim of this bachelor thesis is to develop and validate a software tool able to obtain APDs and CV from intracardiac EGM signals recorded directly from a swine heart. Electrical recording results are validated with optical mapping (OM) data recordings. The code was implemented with the purpose to work for nearby sinus rhythms, that is when it can be useful in clinical application to identify in patients with paroxystic AF the regions associated to the trigger of the AF.

Simultaneous electrical and OM recordings of isolated swine heart are performed in a Langendorff system set-up. Software code processes electrical recordings by computing APDs with Botteron algorithm and computing phase maps. Once phase maps are obtained, trajectories are computed and thus CV. Electrical results are validated with OM data. Two catheters of different sizes are tested as well as results using 15 or 5 out of the 15 electrodes available in each catheter.

APDs results were highly accurate, they were very similar to the OM data independently of whether catheter or number of electrodes was used. CV results were also similar to the OM data, varying inside the acceptable range proposed. Best results of CV were obtained with the small catheter and specifically for 5 electrode interpolation. Both APDs and CV results did not show statistically significative differences with the analysis of the T-student test.

It can be concluded that the software code developed in this thesis demonstrates that is possible to compute both APD and CV values from intracardiac EGM signals with an acceptable accuracy. Optimal results for CV computation were obtained with the small catheter (2.5 cm radius) and using only 5 out of the 15 electrodes. This opens new insights into the use of EGM signals for the clinical estimation of proarrhythmic areas.

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List of Abbreviations

AP	Action Potential
APD	Action Potential Duration
AF	Atrial Fibrillation
AV node	Atrioventricular Node
CCD	Charged Coupled Device
CV	Conduction Velocity
ECG	Electrocardiogram
EGM	Electrogram
HT	Hilbert Transform
OM	Optical Mapping
PVI	Pulmonary Vein Isolation
SA node	Sinoatrial Node

Introduction

Cardiovascular diseases are the major cause of death worldwide [1] causing around 801,000 deaths in United Stated (US) and more than 1,8 million deaths in the European Union (UE) (accounting for 45% and 37% of all death respectively) [2], [3]. Not only a health matter but also an economic issue: the total budget spent because of cardiovascular diseases by year ascended to approximately €210 billion in EU [2] and $316 billion in US [3]. Arrhythmias are a type of cardiovascular disease where the normal heart rhythm is altered. Atrial fibrillation (AF) is the most common type of arrhythmia where the heart rhythm is increased and electrical activation in atria is produced randomly [4].

AF affects about the 2% of the global population: over 6 million people in Europe [5] from which 1 million affected only in Spain [6]. Moreover, AF incidence is expected to keep increasing: after a 13% increase in the last two decades [5] it is expected to be doubled in the next 50 years [7] (due to the increased elderly population). On top of that, the presence of AF doubles the mortality probability [8]. Thus, investigation is crucial for both more specialized and better treatments and for an improvement in prevention. Currently there are research lines that are putting a lot of effort on AF investigation.

Mechanism underlying the trigger and the maintenance of AF are still not clear. Different hypothesis state diverse approaches to understand it [9], [10]. However it is clear that specific biomarkers are related with the AF: such as slow conduction velocities (CV) [11], [12] and shorter action potential duration (APD) [13], [14].

Intracardiac electrogram (EGM) signals recorded with catheters might be utilized in electrophysiology as an assessment tool and, moreover, further analysis might provide information about AF targets during ablation procedures (the ultimate treatment for sustained arrhythmias). The problem is that intracardiac EGM signals are complex and its interpretation is laborious. Considerable approaches have been done in this field [15], [16]. However, the lack of an standard protocol and a correct validation of those analysis lead to unrelated results [17].

This bachelor thesis aims to develop and validate a software tool to analyze intracardiac EGM signals and obtain biomarker measures, as APDs and CV, providing relevant information about AF initiation and maintenance.

Objectives

The main goal of this bachelor thesis is to develop and validate a signal processing tool able to facilitate the interpretation of intracardiac electrograms. Specifically, the major aim is to define the process to easily obtain phase maps, action potential duration (APD) and conduction velocities (CV) of the heart based in an intracardiac electrogram (EGM) single catheter. All phase maps, action potential duration and conduction velocities provide relevant information related to the trigger and maintenance of atrial fibrillation (AF).

A novel signal processing tool will be developed and validated by using as gold standard optical mapping data in isolated porcine hearts perfused in a Langendorff system. Electrical and optical mapping recordings will be performed simultaneously to ensure the validation process of the software created.

The process followed in the development of this bachelor thesis may be summed up as:

- Bibliographic study and review of existing literature related to atrial fibrillation and more specifically about interpretation and obtainment of intracardiac EGM.

- Data recording from isolated swine heart experiments:

    - Preparation and setup.

    - Protocol development: catheter, pace rate and recording mode selection.

    - Development of an optimized protocol.

- Development of the signal processing software for the analysis of intracardiac EGM signals:

    - Phase maps achievement.

    - Action Potential Duration calculation.

    - Conduction Velocity calculation.

- Validation and interpretation of results.

Background

This section is aimed to review the theoretical fundamentals in which this bachelor thesis has been sustained. This project is intended to develop a software tool to analyse biological data so both clinical and technical background will be explored.

Heart anatomy & physiology

The heart is an organ located in the middle region of the thoracic cavity. It is composed of four chambers: left and right atria, and left and right ventricle Figure 1. Atria and ventricles are separated by the intraventricular septum. Its main function is to pump blood to the whole organism supplying both oxygen and nutrients to the entire body and assisting in removing metabolic wastes as well [18].

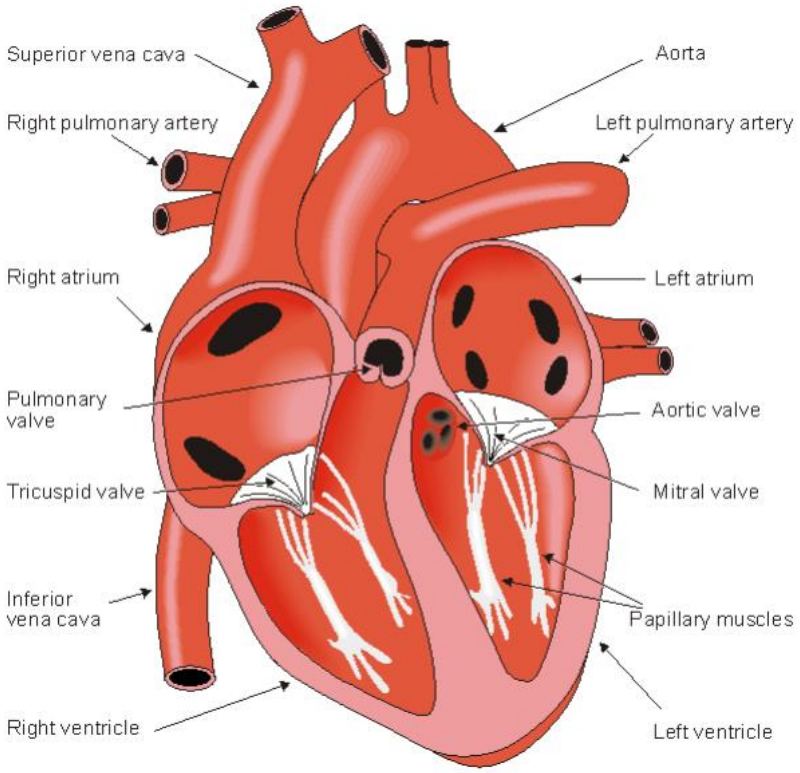

Figure 1. Anatomy of the heart [18].

Heart pumps blood through two different circulatory systems: systemic system (left side) and pulmonary circuit (right side). The first one receives oxygenated blood from the lungs and pumps it to the whole body. The second one, the pulmonary circuit, receives deoxygenate blood from the body and pumps it to the lungs to be oxygenated [19].

Contraction of the heart, and thus pumping of the blood, is produced because of the electrical stimulation of cardiac cells called cardiomyocytes. The heart rhythm is self-paced due to its inherent and rhythmic electrical activity. It is crucial to maintain the rhythm on the conduction system to ensure that the atria and ventricle contract in a coordinated manner and avoid irregular beating of the heart that can result in arrhythmias (abnormal beating) [20].

Under normal conditions, the conduction system works as shown in Figure 2. Stimulus arises from sinoatrial Node (SA node) (located in the right atrium) which is referred as the pacemaker of the heart. It spontaneously generates electrical impulses that spread across both left and right atria resulting in atrial contraction (atrial systole, ventricular diastole). Then the impulse reaches the atrioventricular node (AV node), located on top of the intraventricular septum, that grant the conduction pathway between atria and ventricles.

Next, impulses pass into the AV bundle or bundle of His and to the respective Purkinje fibers of each side of the heart that are able to rapidly transmit cardiac impulses from the bundle to the myocardium of ventricles. Ventricular contraction (ventricular systole, atrial diastole) is enabled thanks to this rapid conduction and blood exits the ventricles via pulmonary artery and aorta.

In case the SA node is damaged or malfunctioning, AV node cells would act as pacemaker. In the event of lack of coordination between both or malfunctioning an abnormal beating, an arrhythmia, is expected. Also, problems in the conduction system (as in the case of scars, anatomic obstacles) would lead to the appearance of an arrhythmia (that will be discussed later).

Cardiac electrical activity is the result of the propagating action potential in cardiac cells. Changes in the ion concentration across cardiomyocytes cell membrane results in changes in membrane potential which are the driver of the action potential. Hence, repolarization and depolarization of membrane potentials are the reason of electrical conduction in the heart. While action potential spreads through the heart electrical currents that can be detected at the surface of the body are generated.

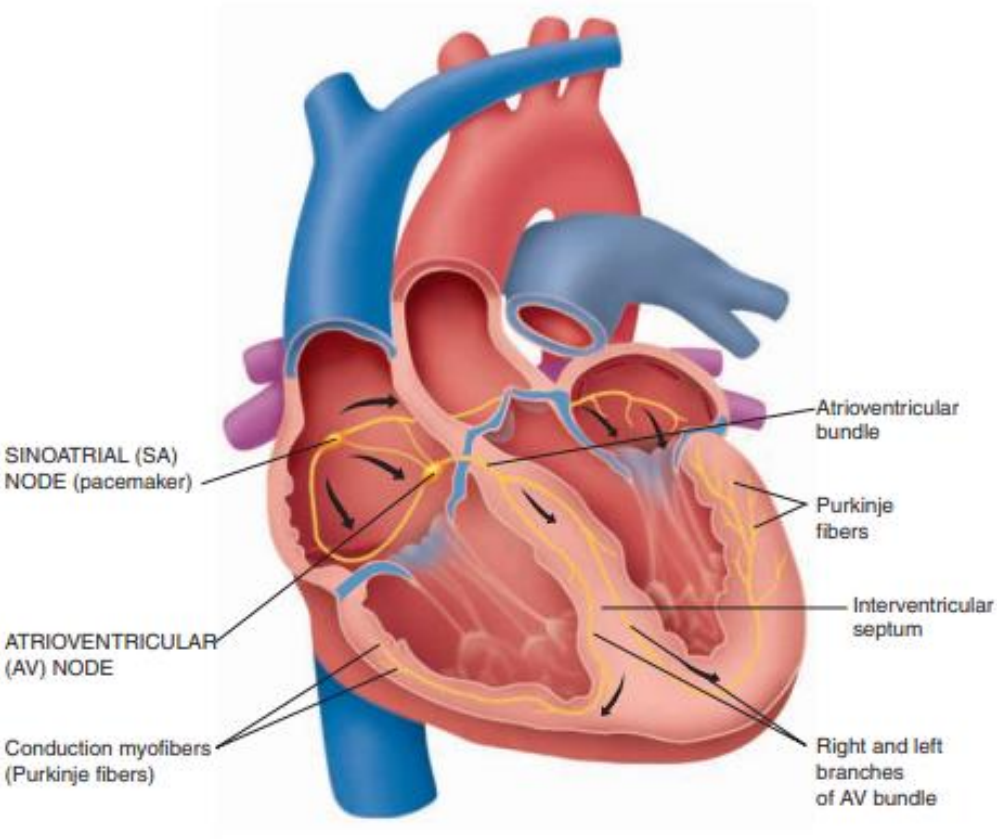

Figure 2. Conduction system of the heart. Impulse arises from the SA node and arrives to the AV node. Contraction of the atria is followed, under normal conduction, by the contraction of the ventricles [20].

Cardiac electrical activity is the result of the propagating action potential in cardiac cells. Changes in the ion concentration across cardiomyocytes cell membrane results in changes in membrane potential which are the driver of the action potential. Hence, repolarization and depolarization of membrane potentials are the reason of electrical conduction in the heart. While action potential spreads through the heart electrical currents that can be detected at the surface of the body are generated.

An electrocardiogram (ECG/EKG) is the recording of these electrical signals and its morphology provides useful information of the overall cardiac electrical activity. The PQRST complex is the signal resulting from the ECG recording. Characteristic morphology of this signal provides information about the electrical status of the heart. Atrial depolarization coincides with the initial P-wave of the ECG. Depolarization of ventricles results in the QRS complex. (atria repolarization occurs at the same time but is not visible in the ECG recording). Final T-wave corresponds to the ventricle repolarization. The resulting PQRST

complex registered in the ECG is shown in Figure 3, as well as the multiple signals from the conduction pathway of the heart that conforms it.

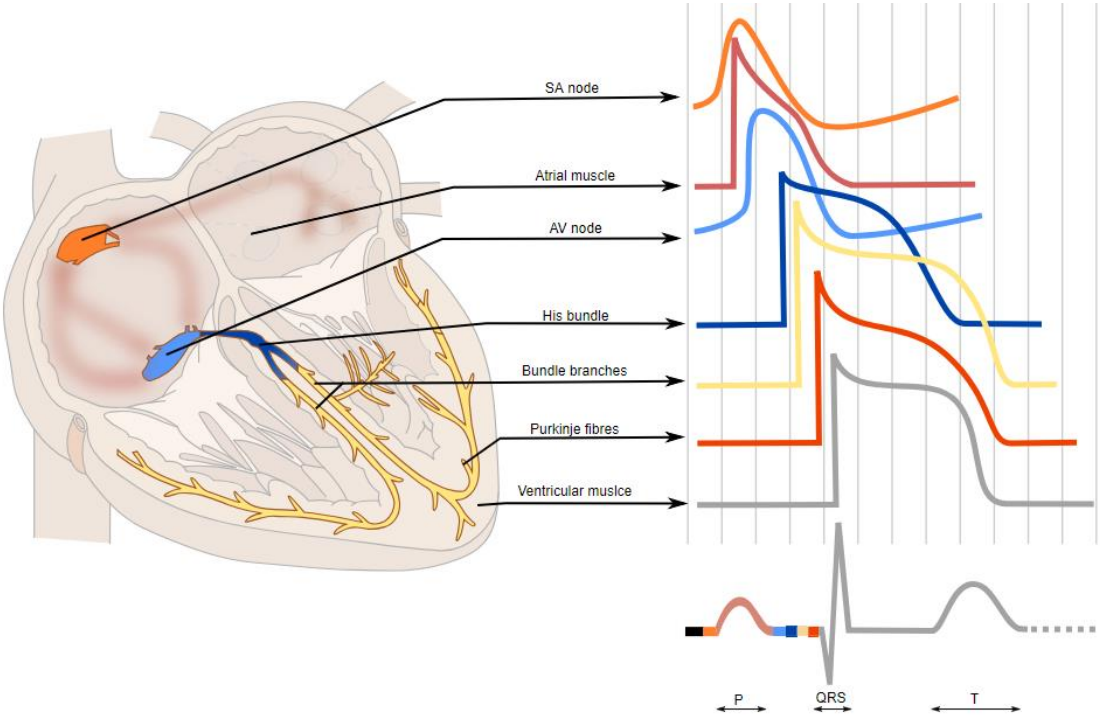

Figure 3. Electrical signals in the different points of the conduction pathway of the heart and the resulting PQRST complex registered in an ECG [21].

Arrhythmias: Atrial Fibrillation

When normal sinus rhythm of electrical activity is disrupted, the heart suffers a cardiac arrhythmia. Heart beating may be accelerated, decelerated or irregular during an arrhythmia. Rhythm disturbance may be a consequence of disorders in impulse formation or conduction or a combination of both [18].

Arrhythmias may be classified according to cardiac frequency into tachyarrhythmias (accelerated rhythm, over 100 beats per minute) or bradyarrhythmias (decelerated rhythm, less than 60 beats per minute) [22], [23]. Firsts are much more common and can be in turn subdivided into supraventricular and ventricular tachyarrhythmias. Among tachyarrhythmias, Atrial Fibrillation (AF) is the most common one [5] and may lead to heart failure, stroke and other complications.

This bachelor thesis focused on the identification of phase maps, APDs and CV, biomarkers related to AF. Current section is aimed to give an overall knowledge about AF to ease the comprehension of the project itself.

Atrial Fibrillation

As previously mentioned, AF is the most common type of tachyarrhythmia and the leading cause of stroke [24], [25]. Mechanisms underlying the trigger and sustaining of this arrhythmia are still not clear and considerable research is being performed to better understand AF. During AF both left and right atria beat irregularly and chaotically as it can be seen in Figure 4 where the impulse arises randomly [26]. This chaotical beating compromises the coordination between atria and ventricles. AP propagation does not arise from the SA node but from other atrial places leading on arrhythmic and fast atrial contractions that are not followed by ventricular contractions.

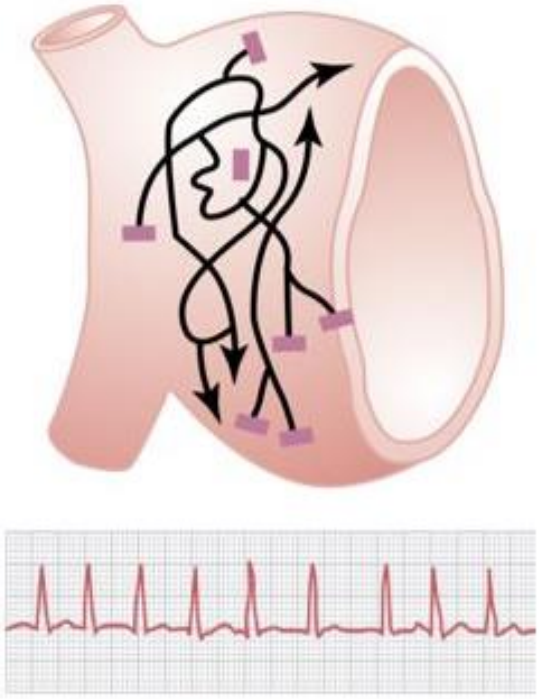

Figure 4. Chaotical activation during AF (top) and resulting AF ECG signal (bottom) [26].

According to the recurrence of AF it can be classified into recently diagnosed (first symptomatic episode), paroxysmal (self-terminating, duration diverges from minutes to

days) and chronic or persistent AF (it lasts over 7 days and does not remit spontaneously) [27].

Mechanism underlying the maintenance of AF is not known yet and several hypothesis states different theories. Main hypothesis can be reduced to three: multiple wavelet, ectopic foci and rotor hypothesis [28] whose mechanism is schematized in Figure 5.

1. Multiple wavelet hypothesis states that several spreading chaotically wavefronts across atria generate AF [9]. However, experimental data and the increasing success in ablation therapies contradicts this theory [28].

2. Ectopic foci hypothesis states that in atrial tissues wavefronts arising from focal sources of high frequencies become fractionate maintaining this way the AF [10].

3. Rotor hypothesis states the AF is an organized process in the spatio-temporal domain and triggered by rotors or functional re-entries at high frequencies focal points that generates turbulent activation in the surroundings. Moreover, this hypothesis for the underlying of AF mechanism is compatible with the presence of multiple wavelets and ectopic foci [28].

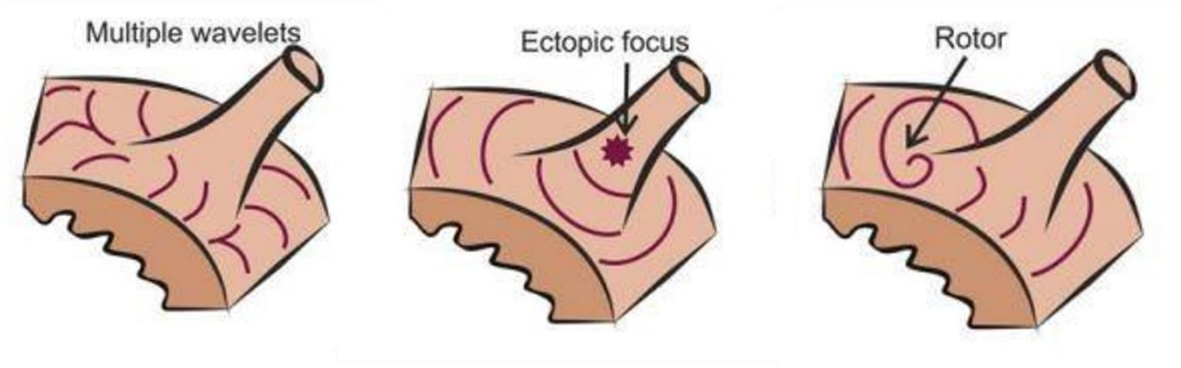

Figure 5. Illustration of the main three hypothesis for AF maintenance. From left to right: multiple wavelets, ectopic focus and rotor hypothesis [28].

Regardless of the different hypothesis, it is known that for an episode of AF to occur, a trigger is needed as well as a substrate that enables the re-entrant activity [9]. Eliminating the trigger may be enough to treat paroxysmal AF. In persistent AF cases the process is more complex because the atrial has undergone a process of electrical and structural remodelling. Changes in atrial tissues modifies its properties turning it into more prone to the sustaining of the chaotic activity [29].

The remodelling process may be due to the underlying cardiac condition, aging or because of the presence of the AF itself [30]. Persistent presence of atrial fibrillation may cause the

appearance of fibrosis due to the reduced contractility [31]. AF causing remodelling favours the increasing nature of tachyarrhythmia.

Among the properties that when modified favour the maintenance of the AF some important biomarkers are found. Shorter AP duration (APD), short refractory period or decreased conduction velocities are an example [32]. It is demonstrated that reduced CV makes the substrate likely to maintain the AF [11], [29]. Specifically, this thesis focuses on the development of a software able to compute atrial CV as an indicator of areas prone to harbour AF.

Atrial Fibrillation: treatment

Current treatments for AF involve both pharmacological and non-surgical interventions [33]. Administration of anti-arrhythmic drugs, electrical cardioversion and catheter ablation are the current methods used to restore sinus rhythm while AF although the success rate of these treatments is not optimal [29], [34].

- Pharmacology therapy: anti-arrhythmic drugs

Anti-arrhythmic drugs are intended to provoke a pharmacological cardioversion, dealing with both the source and the symptoms of the arrhythmia [34] ,[35]. As a prevention also, anticoagulant drugs may be administered in order to prevent blood clots and thus stroke prevention.

- Non-surgical interventions

a) Electrical cardioversion

Electrical cardioversion is a medical non-surgical procedure that aims to restore sinus rhythm of the heart involving the delivery of a controlled electrical discharge through the chest wall producing an electric shock. Result of the cardioversion can be observed in ECG signals as the one in Figure 6, where it is also shown the pre-cardioversion ECG signal and a schematization of the process. Electrical impulse is delivered via a defibrillator [36]. However, this treatment is not intended to solve the source of the arrhythmia so consequently patients can suffer another AF episode in the future.

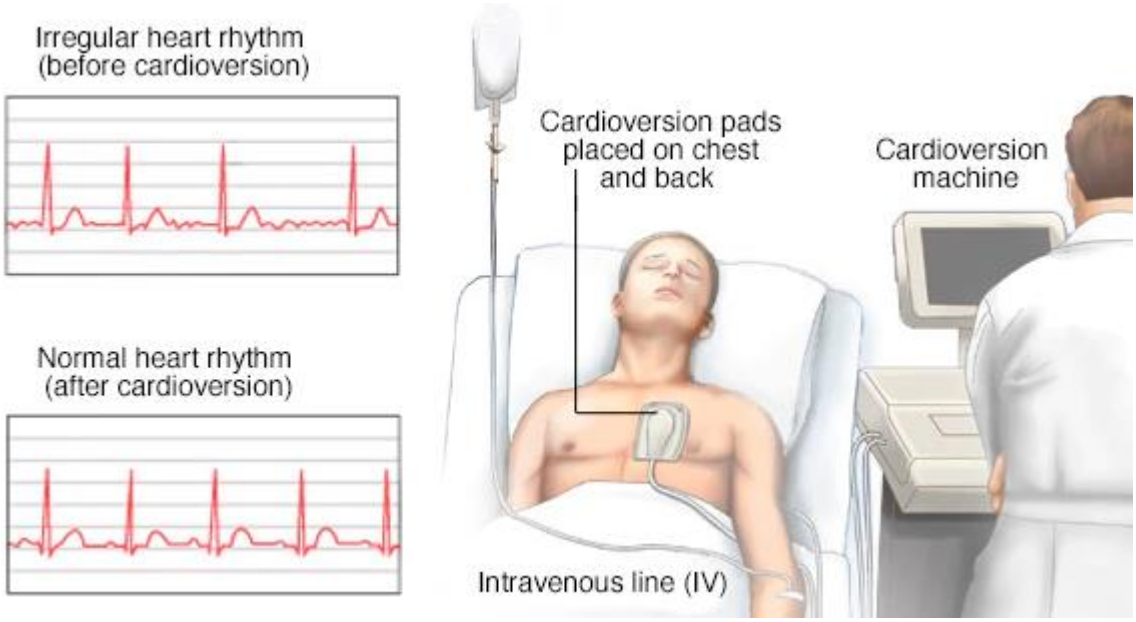

Figure 6. Electrical cardioversion procedure. ECG signals pre- and post- cardioversion are shown (left top and bottom respectively) where it can be seen how the irregular atrium beats stops. A scheme of the procedure including the patient and the operator (right) is represented [37].

.

b) Catheter ablation

In persistent AF when the other treatments are no longer effective a catheter ablation procedure is performed. This one is the most aggressive of the treatments although the most effective one [38]. Radiofrequency catheters are used to isolate via cauterization the region of tissue where the electrical disturbance is supposed to arise of as it is exemplified with two of the ablation catheters available in the market in Figure 7. Despite their higher efficacy their results are not long-term proved and up to 43% of patients have recurrence in the following firsts months after the procedure [28].

Several approaches may be taken to proceed to the ablation but the most common one is the Pulmonary Vein Isolation (PVI) because of its arrhythmogenicity. PVI ablation method consists on atria isolation by ablation around the pulmonary veins [39].

Recent approaches suggest that locating the focal source of AF and using it as a target for this procedure will increase its effectiveness. Moreover, not only effectiveness but also times in the ablation procedure would be reduced which benefit the patient. Both increasing effectiveness (procedures may not need to be repeated) and reduced time impact in the total cost of the procedure, which will be reduced. Intense research is being performed on this subject [40], [41].

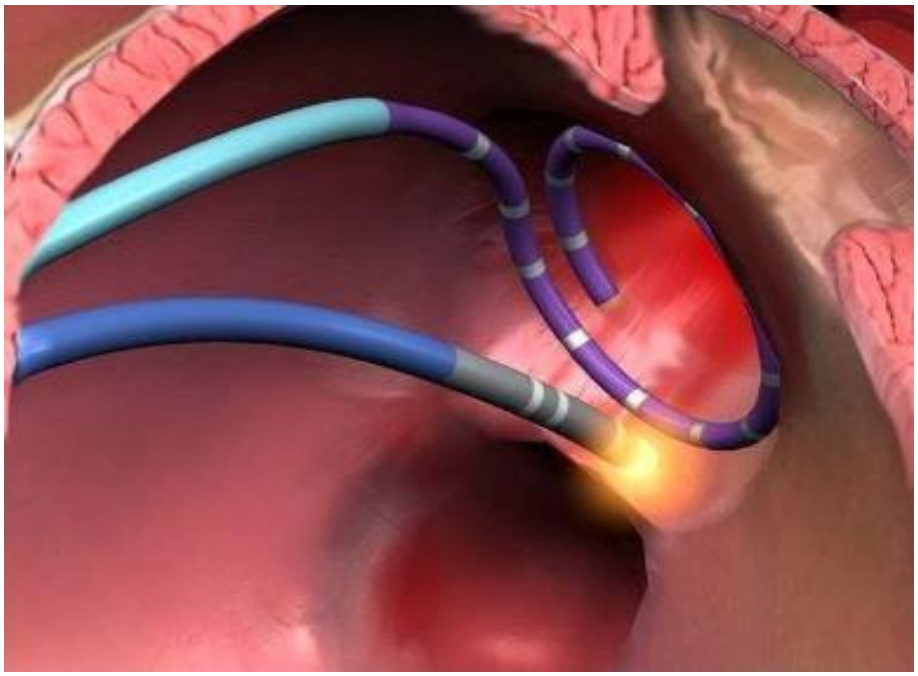

Figure 7. Radiofrequency ablation: ablation catheter (blue straight one) is cauterizing the surrounding area of the pulmonary veins signalized by the mapping catheter (purple circle one) [42].

Cardiac navigation: Intracardiac EGM and catheters

Intracardiac EGM are the record of electrical potential changes (as the previously mentioned ECG), but taking directly from the surface tissue of the chambers of the heart. Recordings are performed through a catheter that is introduced via the femoral vein up to the heart where the registration takes place.

Intracardiac EGM are used as electrical references during electrophysiological studies and may be a very useful tool in guided ablation therapies or for the assessment of cardiac electrical status. Problem arises from its difficult interpretation and its variable morphology depending on recording mode or catheter size and shape utilized.

- ## Analysis of Intracardiac EGM

One of the main approaches utilized when it comes to the analysis of intracardiac EGM is the calculation of the Local Activation Time (LAT) from which biomarkers as CV are later computed. Problem is that LAT calculation is strongly related with the recording mode of

the intracardiac signal: unipolar or bipolar (although investigation with other type of recordings as omnipolar is being performed as well [43]). Unipolar recording allows for an easy calculation of LAT point but has to face with far-field signal issues, whereas bipolar EGM avoids far-field signals but it is orientation-dependant [44]. Time and frequency approaches to analyse intracardiac EGM activation rate are validated with optical mapping (OM) (the same validation that was performed in this thesis) showed that results were unrelated [17].

Although there is no current validated method to compute APD and CV (the ones existing showed to not correlate well with OM data) significative advances are made regarding to processing algorithms for intracardiac electrograms. Most relevant ones include calculation of the phase of the signals since it is a robust method and it does not depend on the amplitude of the signal [45]. To compute instantaneous phase, Hilbert transform (HT) is frequently used [46], [47]. Kuklik et al. developed an algorithm based on wavelets decomposition an Hilbert Transform to accurately calculate in sinus rhythm phase of intracardiac unipolar signals [46]. Phase calculation as a mean to further interpret intracardiac EGM signals as the example of identification of focal points during AF (that are the main target for ablation therapies) in mathematical simulations [48] where it is also proposed a method to be able to interpolate phase signals coherently.

- ## Catheters

Radiofrequency and diagnosis catheters are both intracardiac catheters that can be used during the same electrophysiological intervention. A broad spectrum of both catheters types is available from the principal trading houses as Medtronic, Boston Scientific, Abbott or Biosense Webster.

Specifically, some examples of mapping catheters are shown in Figure 8 . As it can be seen there are multiple possible shapes for the catheter and for its size. Each shape and size can represent an advantage from another one depending on the intended purpose.

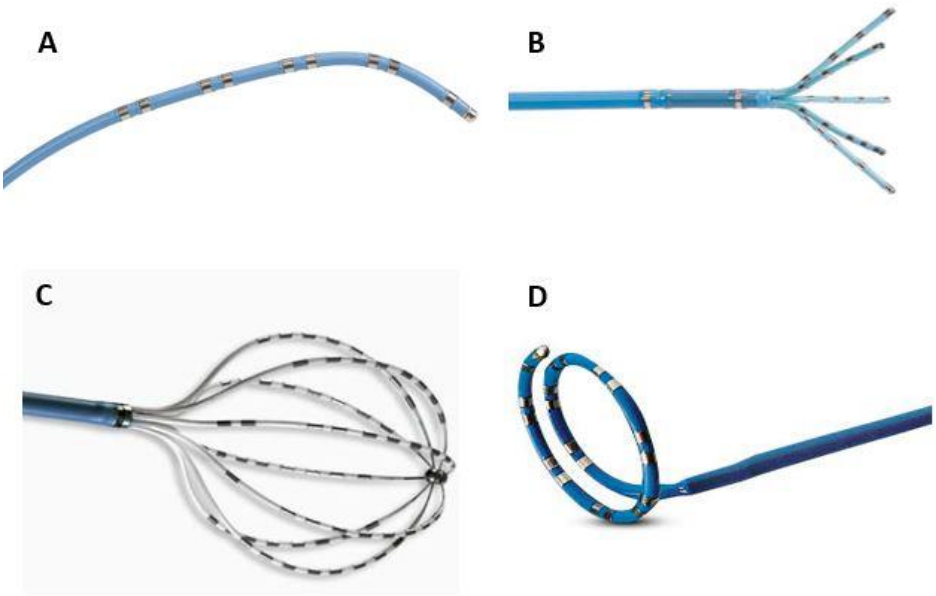

Figure 8. Intracardiac catheters. A: Decapolar (Biosense Webster); B: Pentarray (Biosense Webster); C: Basket catheter (Boston Scientific); D: Spiral Catheter (Abbot).

During this bachelor thesis different catheters were testes but the final results analysed and showed, were performed with two catheters that are being developed and tested in the Laboratorio de Investigación Traslacional en Cardiología del Gregorio Marañón [49]. Specifically, they are two catheters, pentarray-like geometry, with the same shape but in different sizes with five arms and three electrodes each (15 electrodes per catheter) one with an electrode configuration and density as shown in the image below [Figure 9].

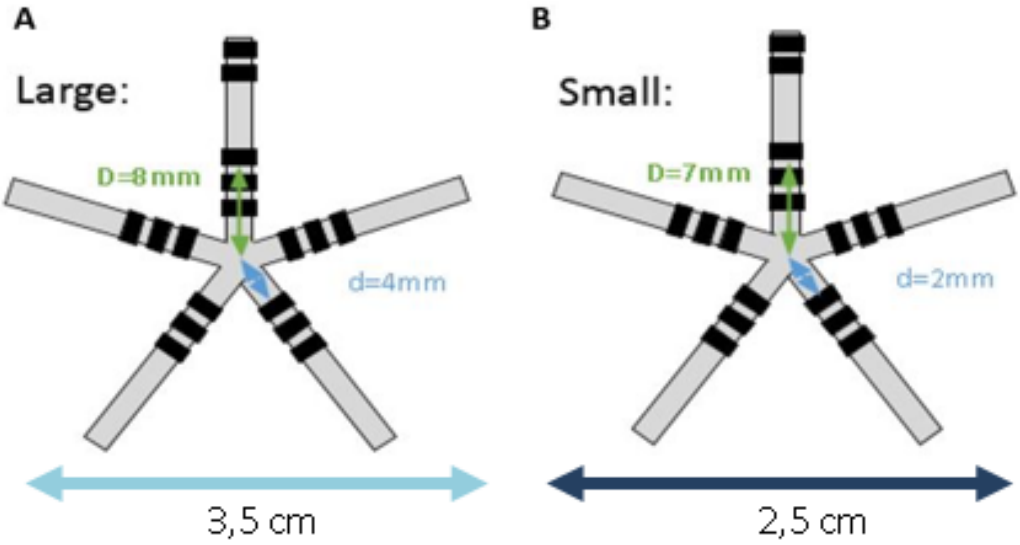

Figure 9. Electrode configuration of the catheters utilized. (A) large size; (B) small size.

AF biomarkers: CV and APD

Alterations of the tissue can lead to modified propagation of the impulse through the cardiac tissue and lead to a reentry around which AF can start. Case of alterations that can alter the normal conduction of the heart and thus are related with AF initiation are slower conduction velocities and shorter APDs [Figure 10].

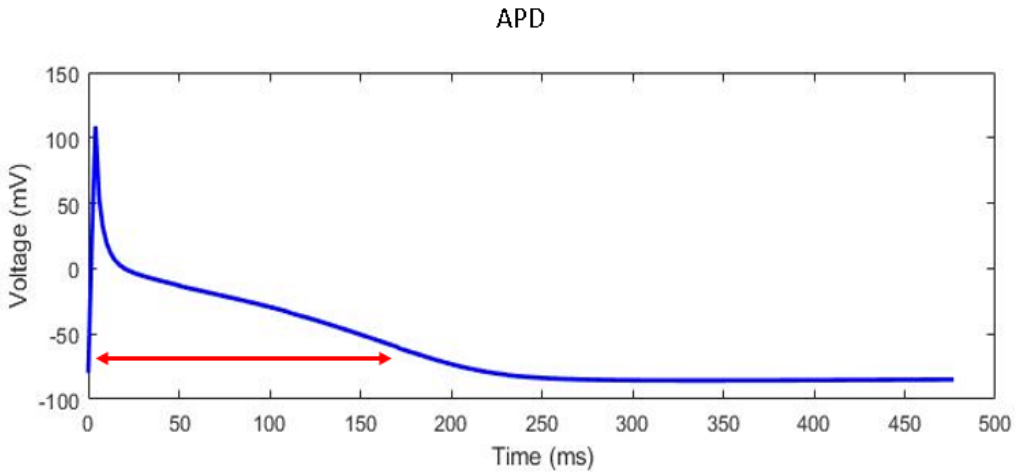

Figure 10. Cardiac Action Potential (blue) and APD (red): interval between depolarization and repolarization of the cell.

Relation between slow conduction velocity is exemplified in Figure 11 from t1 to t4 with the steps leading to a re-entry. When there is a zone of slow conduction in the cardiac tissue (Figure 11, t1), impulse propagates around the slow CV zone and depolarizes faster than slow CV area (Figure 11,t 2). This leads to a situation in which when the slow CV area gets depolarized, impulse cannot propagate straight since it has been already depolarized due to the propagation of the impulse in the normal conduction regions (Figure 11, t3). Consequently, impulse emerging from the slow CV area changes direction and a reentry is produced around the region (Figure 11, t4). So that is why, as previously mentioned slowed CV is associate with a higher probability of arrhythmia [50] and thus it becomes crucial to be able to measure it in patients. Determining areas of slow conduction velocity is key to direct the ablation therapy towards this proarrhythmic area, and therefore improve the ablation therapy efficiency and reduce the time of the intervention.

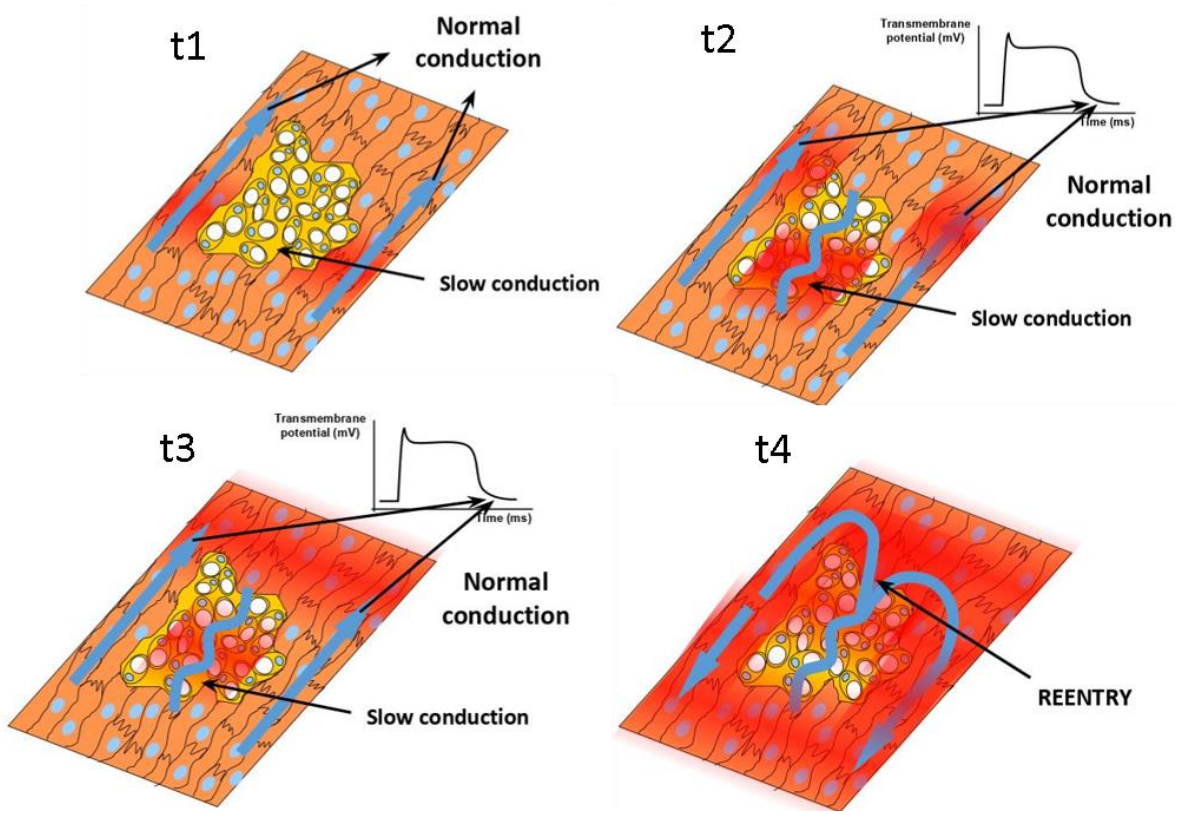

Figure 11. Reentry relation with slow conduction velocity.

The same mechanism relates the shortening of APDs with the formation of re-entries. When action potentials duration is shortened, impulse propagation in the region is faster than usual, an equal region of depolarized and repolarized in the surrounding tissue led to the formation of the reentry around the area of shortened APDs. As in the previous case changes in this value are related to the trigger of an arrhythmia, in this case, shorter APD periods are related to an increased probability of an arrhythmia to occur [51] and thus, it was selected for the intracardiac EGM analysis.

Langendorff system

In cardiac ex-vivo experiments, it is common practice to maintain the organ alive and with its electrical properties intact for the adequate analysis of mechanical and electrophysiological properties. To do so, the organ is placed into a Tyrode-perfused Langendorff system [52], [53] as the one in the Figure 12.

Figure 12. Langendorff system set-up. From left to right: optical mapping system, cannulated isolated heart, perfusion circuit, pumps.

- ## Isolation of the swine heart

Isolation of the swine heart is realized according to the current legislation in the proper facilities intended for that purpose. Once extracted from the animal the organ is immersed into a Cardioplegic solution at 4°C for its cannulation and further transportation.

Cardioplegic solution is intended to induce cardiac arrest by lowering the temperature and reducing cell metabolism to a minimum, therefore preserving the tissue effectively. When immersed in Cardioplegic solution a cannula is introduced in the aorta of the swine heart as in Figure 13. By means of the cannula the organ is later connected to the Langendorff circuit [54].

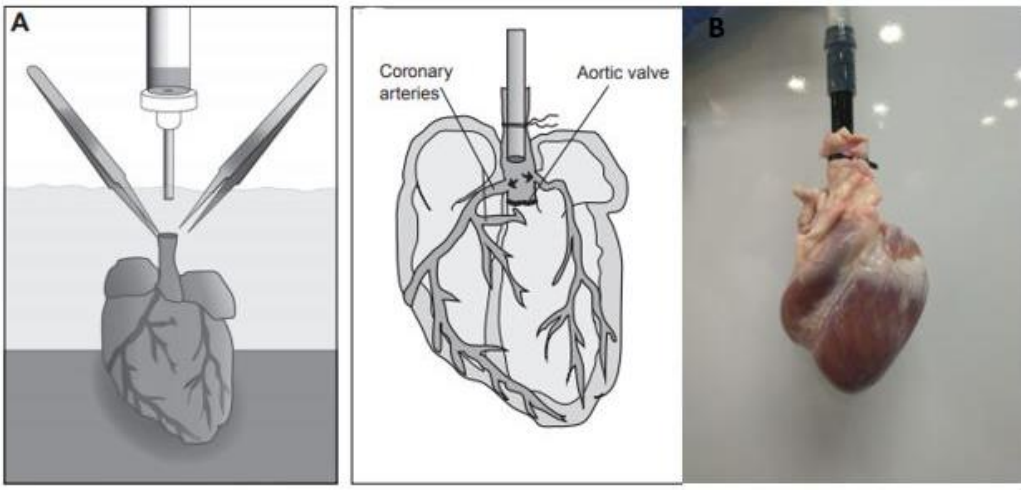

Figure 13. Cannulation of the heart. (A): Cannulation occurs when the organ is immersed in Cardioplegic solution and cannula is introduced in the aorta (middle, right); (B) [54].

- ## Langendorff circuit set-up

Langendorff system is a is a technique that allows the maintenance of an organ ex vivo thanks to perfusion of an oxygenated and rich in nutrients buffer. As previously mentioned for the purpose of this project swine hearts were utilized.

Once the heart is cannulated and located in the Langendorff set-up, it is perfused in a reverse fashion (regressively down the aorta). Due to the retrograde perfusion Tyrode flows through the coronary arteries. Retrograde perfusion may be performed in two modes: constant pressure or constant flow and therefore different pumps may be utilized.

A crucial parameter for heart stability is temperature. The perfusate must be at a constant physiological temperature of 37°C. Heat pumps or heat baths might be used among others. Not only at a constant temperature but at physiological pH: both perfusate and Cardioplegic solution must be pH-adjusted prior of the beginning of the experiment.

Variation of physiological parameters alters the heart's normal behaviour and therefore may lead to a misinterpretation of results.

- ## Perfusate

Different buffer solutions may be used as a perfusate in the Langendorff system. In the case of Langendorff heart experiments Tyrode is usually utilized.

Tyrode is a buffer solution containing glucose, sodium, calcium, magnesium and some other components that provides the heart with nutrients and electrolytes necessaries for its proper functioning. Tyrode is pH-adjusted and then heated once introduced in the circuit thus the perfusate reaches the physiological conditions of the heart.

- ## Oxygenation of the perfusate

Perfusate utilized in the Langendorff system must be adequately oxygenated so the heart receives its proper oxygen intake. An oxygenator is utilized for this end and usually carbogen mix is selected. Depending on the oxygenator election it may work as not only an oxygenator but also a filter in order to ensure the optimal conditions of the buffer arriving to the heart.

The presence of air bubbles could cause irremediable damage to the heart therefore several bubble traps are placed in the circuit: after the oxygenator and in the cannula inserted in the aorta as an ultimate barrier for the air.

- ### Pacing and ECG assessment

Through the use of different catheters, it is possible to both monitor (electrocardiogram register in an oscilloscope) and pace the heart. Usually the heart once perfused naturally beats in sinus rhythm although it not may occur and a defibrillator may be used. Once the mechanical activity of the heart is stopped (as it will be explained later) and the pacing protocol starts.

Optical mapping

Optical mapping (OM) is an imaging technique that utilizes voltage-sensitive or calcium-sensitive dyes to obtain information about transmembrane voltage changes and calcium transients with high spatiotemporal resolution. It is possible to perform optical mapping studies from single cells (in vivo) to whole hearts (ex vivo). It eases the understanding of electrophysiological properties of cardiac tissue and cardiac function itself. Further study of the data obtained provides information about electric propagation, allow to understand the mechanism underlying cardiac arrhythmias and is also used in the development and assessment of different treatment strategies of cardiovascular diseases among other uses [55].

OM is a validated technique with enormous relevance in basic research [53] that allows the performance of simultaneous electrical recordings hence its use as the gold standard for this thesis. Because of the great importance of this technique in the validation of the results, in the following paragraphs it will be explored more in detail.

To carry out optical mapping experiments a set up made of a light source and detector (schematized in Figure 14) as well as filters and fluorescent dyes is needed.

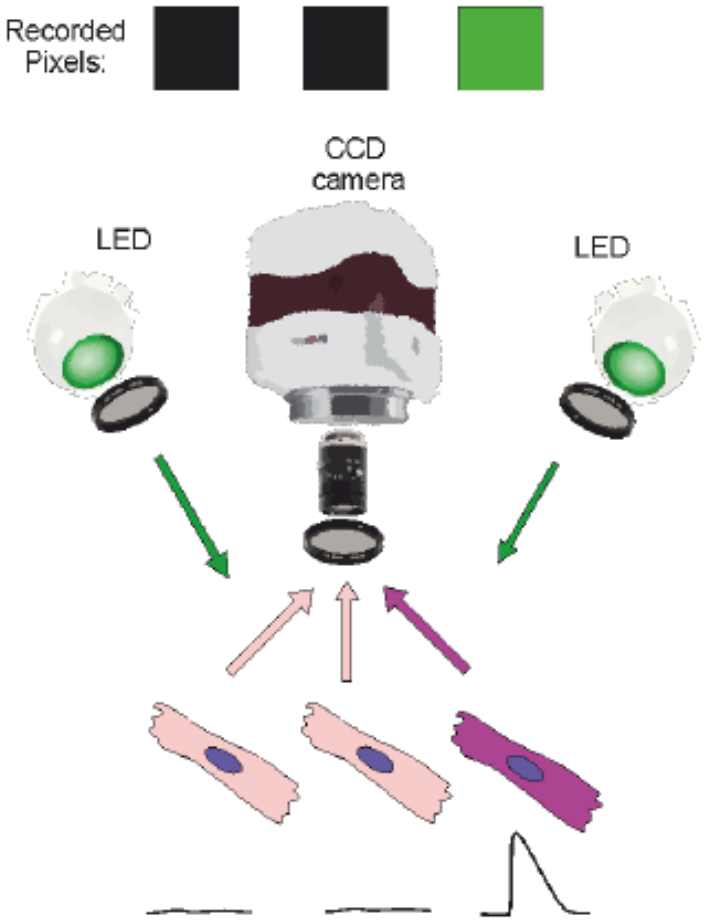

Figure 14. OM light and camera set up.

The basic functioning of the OM experiments is as shown in Figure 15. A voltage-sensitive dye is utilized. The dye binds to the membrane of the cardiomyocytes of the heart. The heart is illuminated with a light source with a specific wavelength corresponding to the absorption spectrum of the dye in order to excite it. Photons are emitted from the dye when returning to ground state. Emitted photons are filtered and redirected to the detector. The extent of emitted photons is quantified for each pixel of the detector as a measure of the membrane potential changes in the heart.

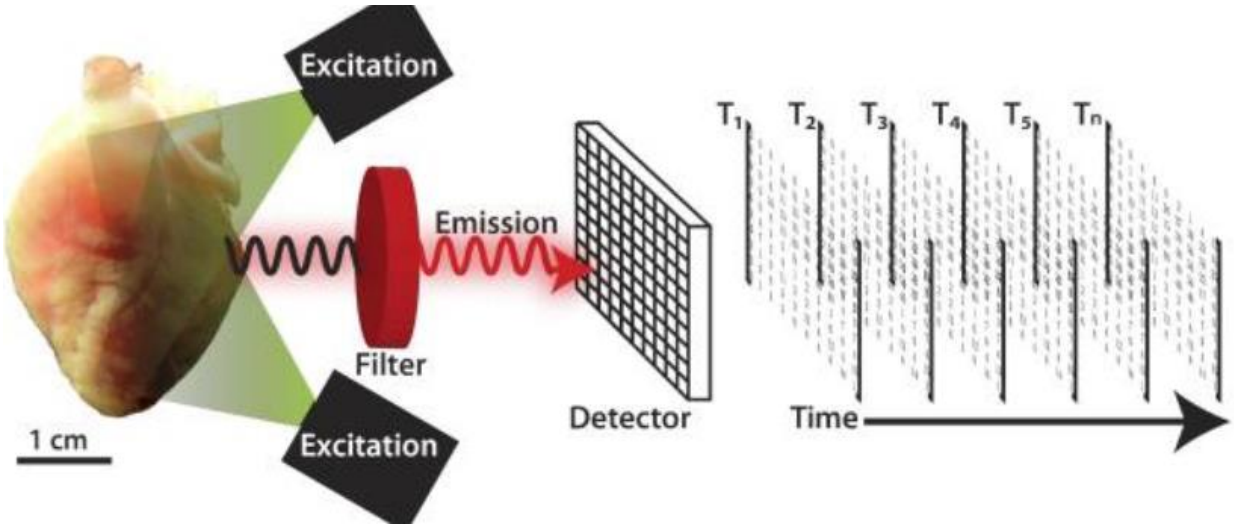

Figure 15. Scheme of the OM set up and data acquisition. Excitation light hit up onto the dyed heart. Once excited the dye bounded to the cell membranes of the organ emit photons that are filtered and redirected to the detector that registers sequentially in time [55].

- **Motion artefact**

When dealing with optical mapping of the heart an important issue must be considered: motion artefact. Due to the intrinsic movement of the heart signals recorded in optical mapping would be affected with this disturbance. To cope with this artefact, an electromechanical uncoupler (it prevents the mechanical movement of the heart unaltering the electrical conduction) might be administered to the heart and/or custom pre-processing treatments to the signals must be applied.

- **Fluorescent dyes: Potentiometric dyes**

Two types of fluorescent dyes are utilized in optical mapping: Potentiometric dyes, which accounts for changes in membrane potential, and Calcium sensitive dyes, which are used as indicator of cardiac excitation-contraction coupling (Calcium plays a main role in this coupling). Due to the rapid optical response of voltage-dependant of fast-voltage sensitive dyes, APD can be measured and thus they are utilized in cardiac electrophysiology [56]. As in this thesis optical mapping was APD calculations and voltage-related information such as the CV of the impulse just potentiometric dyes are briefly reviewed [57].

Potentiometric dyes can be used to measure membrane potential values in whole hearts [58]. These dyes have the characteristic of linearly changing its fluorescence as membrane potential varies (within the physiological parameters) [59].

The mechanism of action of voltage-sensitive dyes is explained by two theories: electrochromic and solvatochromic theory. First one states that an alteration in the electronic transition is produced due to an electronic redistribution whole excitation of membrane potential [60]. The solvatochromic states that the dye molecules suffer a change in polarity (and they are reoriented in the lipid environment) due to an electric field [56].

Thus, depending on the mechanism of action and response time potentiometric dyes are divided in fast-response (electrochromic mechanism) dyes and slow-response (solvatochromic mechanism) dyes [57]. Because of its fast response (order of microseconds), fast-response dyes are utilized in cardiac electrophysiology. Specifically, the most common ones are styryl dyes. Example of styryl dyes widely utilized in cardiac electrophysiology studies is the di-4-ANEPPS. This dye decreases its fluorescence emission in response to increases of membrane potential (hyperpolarization) when excited at proper wavelengths. Di-4-ANEPPS also present high signal to noise ratio (SNR) and respond to short excitation wavelengths [56].

- ## Light source

Proper illumination, stable and uniform, at the correct wavelength is key to success in optical mapping experiments [57]. Lately the use of light-emitting-diodes (LEDs) in confrontation with traditional light sources (xenon, mercury or halogen maps) is used for cardiac optical mapping. They offer more stability and some advantages as energy efficiency, portability flexibility and lower costs [61]. Adequate cooling and output power are some concerns related to the used of LED illumination [57].

More intense excitation produces larger emitted fluorescence which results in larger signals. However, continuous illumination may result in photobleaching so the dye is only illuminated (and therefore excited) the necessary recording time [62] (usually optical mapping experiments are performed in darkness with the exception of the LED illumination when exciting).

- ## Filters

Filtering is aimed at reducing background light and to only let selected wavelengths reach the detector. Excitation and emission filters are used. Excitation filter only allows the

exciting dye wavelength to pass while it blocks emission light at that specific wavelength [56]. High-quality filters also reduce the possibility of the photobleaching effect. They are intended to better select the wavelength spectrum and to obtain better and with high quality images.

- ## Detectors

Photodetectors in cardiac optical mapping must be high-speed (because of the speed propagation of the electrical impulse) and low-noise (because of the weak signal strength produced by voltage-sensitive dyes). Detectors commonly used in cardiac mapping that fulfil this high spatiotemporal resolution criteria are: photodiode arrays (PDAs) and Charge Couple Device (CCD) cameras [57].

CCD cameras offer higher spatiotemporal resolution than PDA (larger pixel quantity) but slower full-frame-rate in contrast. This slow frame rate might be improved by an increase in pixel binning (more than one-pixel red at a time) but will result in a decrease of spatial resolution. This might be a problem in experiments dealing with monolayer cell culture. CCD cameras derivatives are developed to avoid this concern and provide more sensitivity with lower noise: electron-multiplying CCDs (EMCCD) and intensified camera systems [61].

In the case of this thesis, the Optical Mapping set-up had EMCCD as a detector. This technology makes use of impact ionization (avalanche process) prior to the conversion to an electric e signal to produce secondary electrons. The modification to the CCD camera resulting overall better efficiency [63].

Materials and Methods

The current section is intended for the description of the materials used and methods followed during the development of this bachelor thesis. As previously mentioned, the aim of this project is to develop a software tool able to compute conduction velocities and action potential duration from intracardiac electrogram signals. To do so and to perform an optimal gold standard verification, electrical signals are recorded simultaneously along with optical mapping ones. Both registers are further analysed independently and contrasted to assess the efficacy of the elaborated software.

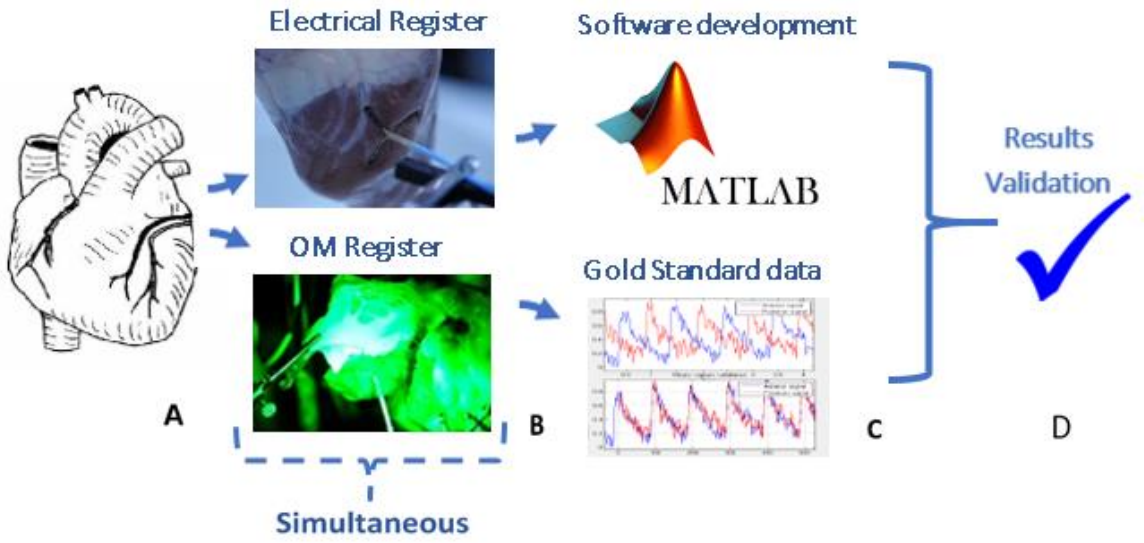

Figure 16.Overall briefed workflow of the project: (A) Isolation of swine hearts and experimental set-up; (B) Simultaneous electrical and optical mapping registration of cardiac electrical activity or the heart; (C) software development and standard gold data collection; (D) Evaluation and validation of the results obtained.

In Figure 16 the overall workflow of the project is summarized. Down below this section the different parts represented in the scheme will be further elaborated.

Experimental set-up

- ## Swine heart isolation

Optical and electrical signals were obtained from an isolated swine heart (race: Minipig) [Figure 17]. Organs utilized in the experiments performed for this thesis were isolated in the Hospital General Universitario Gregorio Marañón, Unit of Experimental Medicine and surgery department. All experiments were approved by the Ethics Committee on Animal Experimentation (EAEC) of the hospital and following the indications of the Helsinki convention as well as the European and Spanish regulations on the subject. In particular, the hearts were isolated by thoracotomy after the general anaesthesia.

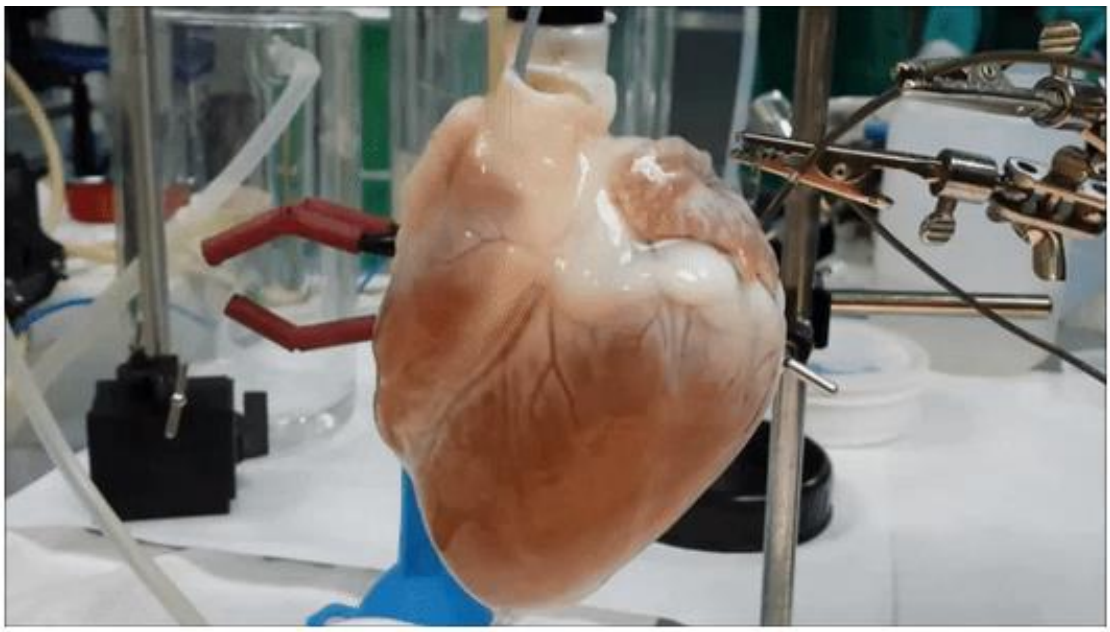

Figure 17. Isolated swine heart.

Once isolated hearts were introduced in Cardioplegic solution at 4°C to cease its activity. While introduced in the Cardioplegic solution were cannulated through the aorta and transported to the lab afterwards. Cannulas were designed in the Laboratorio de Investigación Traslacional en Cardiología using Meshmixer tool, 3D printed and ensembled with the necessary tube and tube connectors.

- **Langendorff set-up**

The cannulated organs were placed into the Langendorff set-up. Swine hearts were continuously perfused with a bubbling carbogen-oxygenated Tyrode solution of 95% O_2 and 5% CO_2.

An oxygenator [Figure 18 A] D 901 Lilliput: Hollow Fibre Oxygenator with Twin Reservoir was utilized along two peristaltic pump Masterflex L/S model number 7524-45, 10-600 RPM assembled in USA by Thermo Fisher Scientific, each one for the incoming and outcoming circuit respectively. Input flow was set to 170 ml/min and output flow to 200 ml/min. A water bath [Figure 18 B] from Anova Industries, Inc,A&C Model, series- E of 220 volts, 50 Hz and 1000 watts was utilized to enable a nearby physiological temperature of 37°C in all circuit.

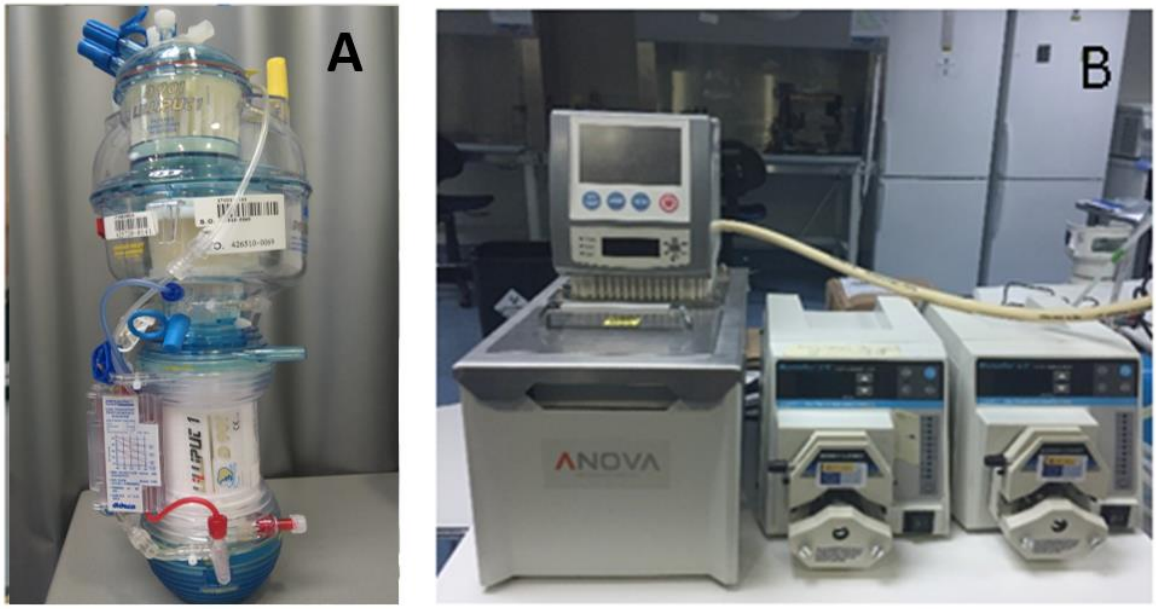

Figure 18.(A) Oxygenator; (B) (from left to right) Water bath and peristaltic pumps.

Data acquisition: simultaneous recordings

Data collection of this thesis was obtained from swine isolated hearts perfused in Langendorff system experiments. Experimental data collection was integrated as a part of the development of the experiments for a larger project being carried out in the *Laboratorio*

de Investigación traslacional en Cardiología of the Hospital Gregorio Marañón. Protocols for both projects were joint together in order to develop the experiments jointly as they did not interfere one with each other (the protocol for this thesis was designed to be developed in non-fibrillation rhythms while the second protocol was intended for AF measurements).

A total of 7 experiments were performed for the development of this thesis. Both the software and the experimental protocol suffered an implementation process that resulted in the fulfilment of this project's objective. Results presented in this thesis are the ones obtained with the data recorded in the last experiment, technical problems with some equipment utilized made invalid signals so the number of results presented in this thesis is reduced.

- ## Optical mapping register

Hearts were infused with 100 µL bolus of di-4-ANEPPS (Biotium, Inc. Hayward, CA, USA) of 4.16 mM (in DMSO) applied over 5 minutes following electromechanical dissociation by the administration of 10 mM of 2,3-butanedione monoxime (Biotium) in Tyrode solution.

In order to excite di-4- ANEPPS, hearts were illuminated with a filtered green LED light source: LED: CBT-90-G (peak power output 58 W; peak wavelength 524 nm; Luminus Devices, Billerica, USA), plano-convex lens (LA1951; focal length= 25.4 mm; Thorlabs, New Jersey, USA) and a green excitation filter (D540/25X; Chroma Technology, Bellows Falls, USA). Such light sources were used to achieve homogeneous illumination. In order to excite di-4- ANEPPS, hearts were illuminated with a filtered green LED light source: LED: CBT-90-G (peak power output 58 W; peak wavelength 524 nm; Luminus Devices, Billerica, USA), plano-convex lens (LA1951; focal length= 25.4 mm; Thorlabs, New Jersey, USA) and a green excitation filter (D540/25X; Chroma Technology, Bellows Falls, USA) [64]. Custom software written in MATLAB and based on Micro-Manager Open Source Software was used to perform optical mapping image recording and processing [Figure 19].

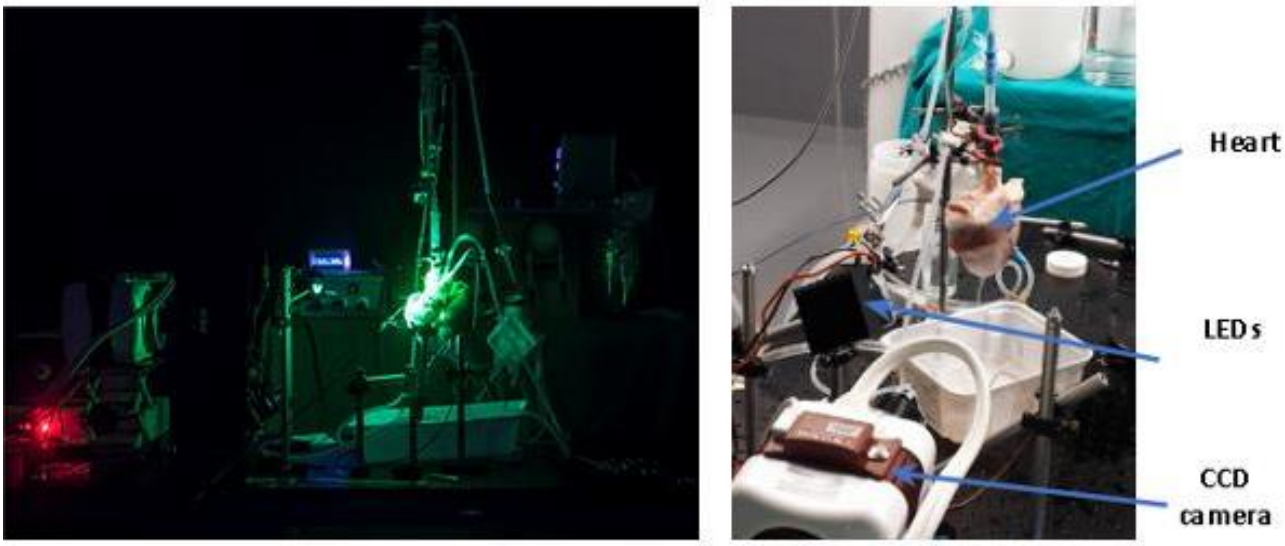

Figure 19.Optical mapping registration process being carried out; (right) optical mapping setup for isolated Langendorff perfused hearts.

- **Electrical register**

Electrical recordings were initially performed with a Decapolar St. Jude catheter [Figure 9 , A] performed on the isolated organ with different types of intracardiac catheters that are being develop in the laboratory [Figure 20 B], [49].

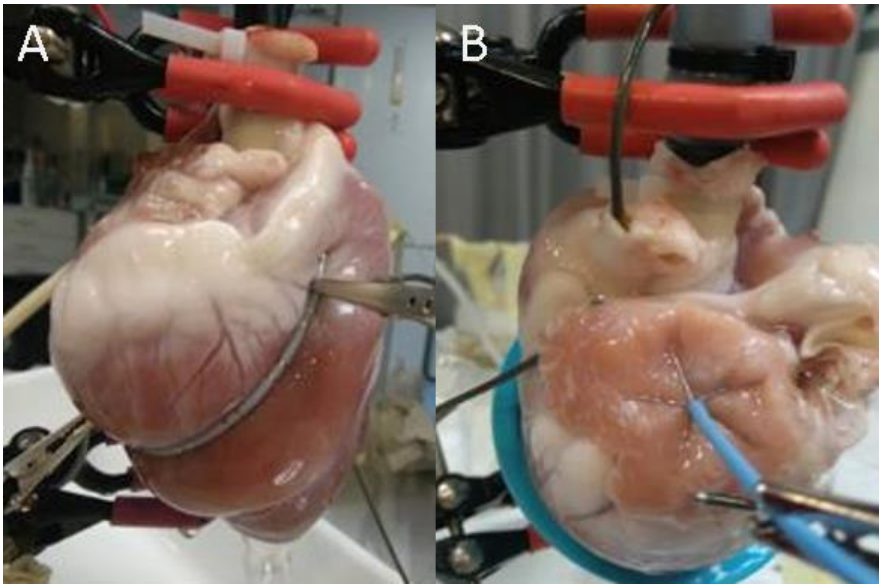

Figure 20. Intracardiac EGM catheters: (A) St. Jude Decapolar; (B) 5-arm laboratory catheter.

Two five-arm catheters of different sizes were connected to a 64 channel-polygraph with reference placed in the right ventricular tract (none electrical activity).

Decapolar catheter registers were only performed in the very first experiments, later on because of the better properties offering the pentarray ones replaced it. Reasons of the mentioned catheter include geometry of the pentarray (it better fit atria, the region of interest) and it lends itself for a better interpolation (further details are provided in the software development section).

Because of the different morphology of the atria between the different hearts it was sometimes necessary to dilate the atria for the catheter to fit onto its surface. Dilatation of the atria was performed by intracardiacally introducing and inflating a balloon, as some clinical catheters allow the same procedure.

All electrical recordings were registered in unipolar mode during 10 seconds with a sampling frequency of 1 kHz.

- ### Electrical Pacing and assessment

Two additional catheters were used: one for pacing the heart and another for EGM monitoring, connected to an oscilloscope (this way heart rhythm can be monitored and status of the heart checked) in real time.

Pacing protocol consisted in the application of a biphasic pulse of 4V and varying pacing intervals, ranging from 300 ms to 1000 ms, to ensure that the algorithm works for a physiological range of heart rhythm from the atria epicardium so that the propagation was longitudinal to epicardial fibre orientation.

Software development

In this section the process for obtaining CV and APD will be described step by step. An overall summary of the whole process is shown in [Figure 21]

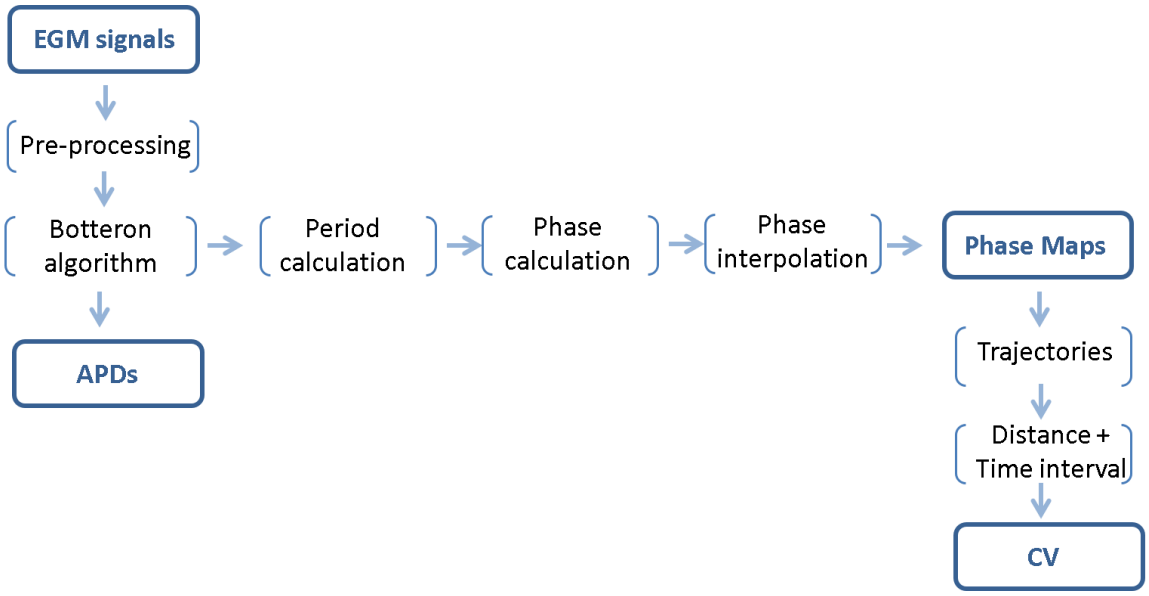

Figure 21. Workflow of the software tool.

- **Signal pre-processing**

Signals were pre-processed in order to remove noise and enhance the features of the signals that were necessary for the further development of the code [Figure 22, A and B]. Pre-processing consisted on drift removal and filtering. To estimate the baseline, ECG signals were decimated to 51.2 Hz and filtered with a Butterworth 10th-order low-pass filter with a cut-off frequency of 2 Hz. Baseline was then interpolated to 1000 Hz and subtracted to the original signal.

- **Botteron Approach**

Botteron approach performs a transformation on the signal in order to emphasise the fundamental frequencies and, in the case of this project, it serves because of two reasons. First it recognizes the depolarization and repolarization along the signal as signalled with the green and red arrow respectively in Figure 22, C. By eliminating the repolarization peak it serves to calculate the period and also the phase (Kuklik's algorithm is applied to Botteron signal after the elimination of repolarization peak). Botteron approach includes absolute value computation and low-pass filtering at 20 Hz.

- ## APD Calculation

From the signals obtained after the application of the Botteron Algorithm, APDs were straight calculated after identifying the peaks present in Botteron signal. By computing the intervals between peaks signalized as in the Figure 22 D APDs were obtained. As previously mentioned, depolarization occurs at different times but repolarization within a vicinity of the tissue occurs at practically the same instant.

- ## Period calculation

Period was computed by performing Welch Periodogram with Hamming window of double length of sampling frequency, i.e. 2000, Fast Fourier Transform of 4096 points and 50% overlap of the Botteron signal (after eliminating the repolarization peaks)

- ## Phase Calculation

Phase is computed using the algorithm proposed by Kuklik et al [46] that is based on the sinusoidal reconstruction (wavelets) of the Hilbert Transform. As an example of the phase signal obtained is showed in Figure 22 E: values in the range of π and -π.

- ## Phase Interpolation

Once phase is calculated the objective was to interpolate the phase data to an 81x81 grid matrix from a geometry created to emulate the actual morphology of the catheter used Figure 22, F. The 81x81 grid was utilized to represent a square of 20x20 mm in 0.25 mm steps where both catheters arms, large and small, were able to fit (r=7mm and r=8mm respectively).

First of all, to interpolate the phase first it was transformed to a complex exponential form as Roney et al. proposed [48]:

$$\theta \rightarrow e^{i\theta}$$

Equation 1. Phase transform.

Once the transformation is performed, the transformed data is interpolated to the 81x81 matrix by a Delaunay triangulation. Then the complex exponential form transformation is undone and phase maps as the ones in Figure 22 G are obtained.

Interpolation is performed from the 15 electrodes available in both large and small catheters and in just 5 out of the 15. Specifically, the five outermost electrodes of each catheter in order to prove the response of the code to both situations and determine the one providing more accurate results.

- ## Trajectory calculation

Impulse trajectory is calculated as an intermediate step between phase maps obtainment and CV computation.

In order to obtain the direction, the 81x81 grid is divided in 5 sections each one comprising one of the catheters legs coordinates.

Once the area has been divided, to achieve better results a frame selection is performed in order to define the pulse intervals when the phase values is varying from -pi to pi. For each of these regions a determined number of time intervals to analyse (each interval corresponds to the depolarization interval of the pulse in the region) is selected.

Afterwards, in each frame of these period intervals the middle point of the depolarizing wave is computed. Summation of all middle points along the different frames of the spreading of this depolarizing wave gives as a result the trajectory of the wave propagation as represented by the red arrow in Figure 22 H.

- ## Velocity calculation

Once the trajectories of the impulse have been calculated, in order to calculate the velocity, middle part of the trajectory is selected and its longitude calculated. Time interval is computed as the time elapsed between the depolarization at the beginning of the trajectory segment and the depolarization of the end of the selected segment. Velocities are then calculated.

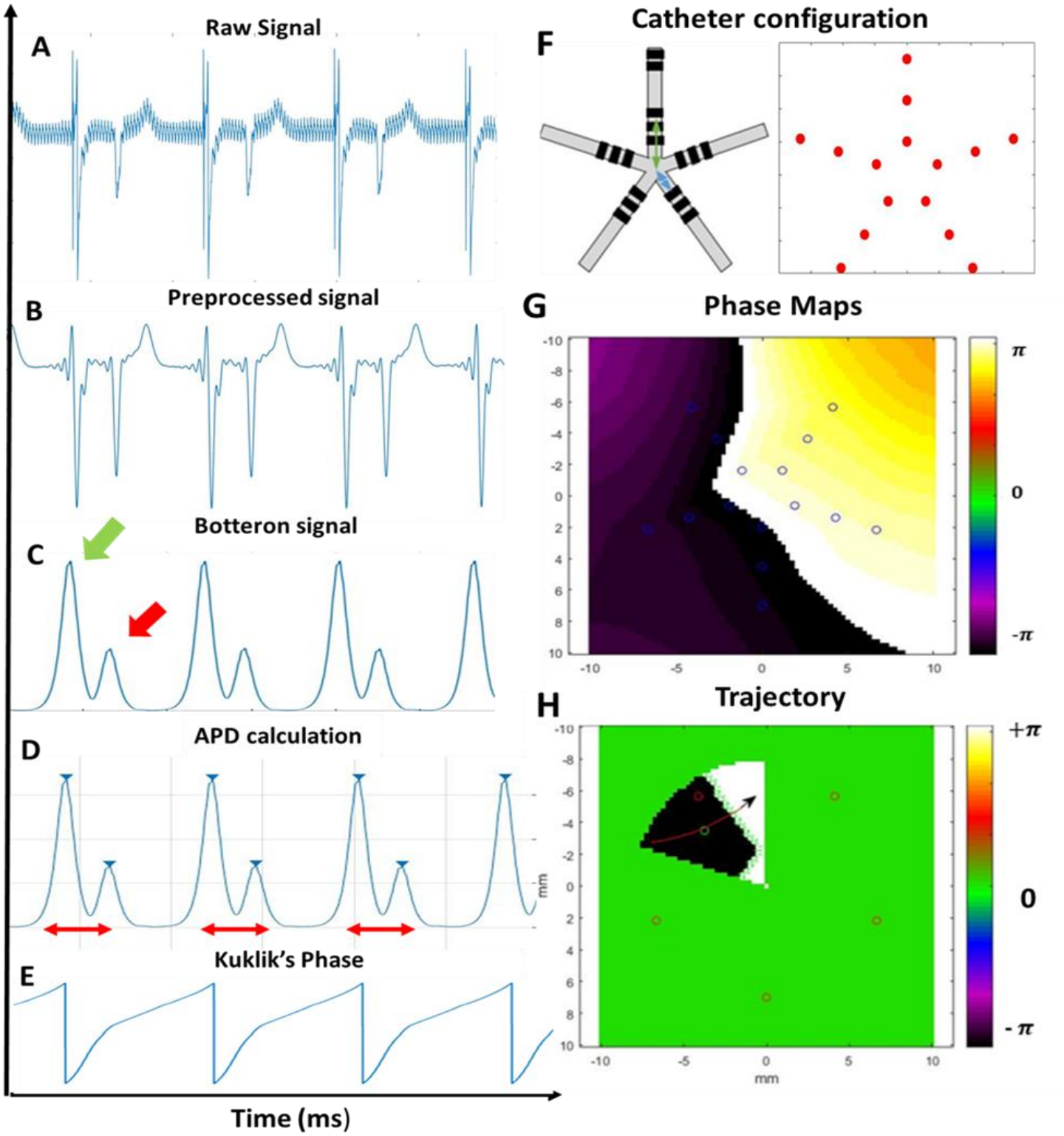

Figure 22. Summary of signal transformation along the software code. (A) Unfiltered Signal; (B) Pre-processed signal; (C) Botteron signal with depolarization and repolarization peaks (green and red respectively); (D) APD computation; (E) Phase signal obtained with Kuklik's algorithm; (F) Catheter configuration simulated in de code; (G) Phase maps obtained in the 81x81 grid; (H) Trajectory computation from phase maps.

Results validation

OM data was utilized as gold standard for this thesis. Both phase maps and CV software results were compared as summarized in Figure 25Figure 24.

Phase map validation was performed as a middle step in the process to ensure that the development of the software was going as expected. Phase maps videos were visually compared with the ones obtained from OM in order to check the accuracy of the method at this step.

With regard of APD and CV values obtainment OM signals were subjected to a pre-processing filter process. Steps followed included: fifth order spatial filtering, eleventh order Sgolay filtering, fifth order time smoothing filtering and baseline removal.

APD calculation was measured by computing the interval between depolarization (upstroke of the stimulus) and repolarization as signalized in Figure 23:

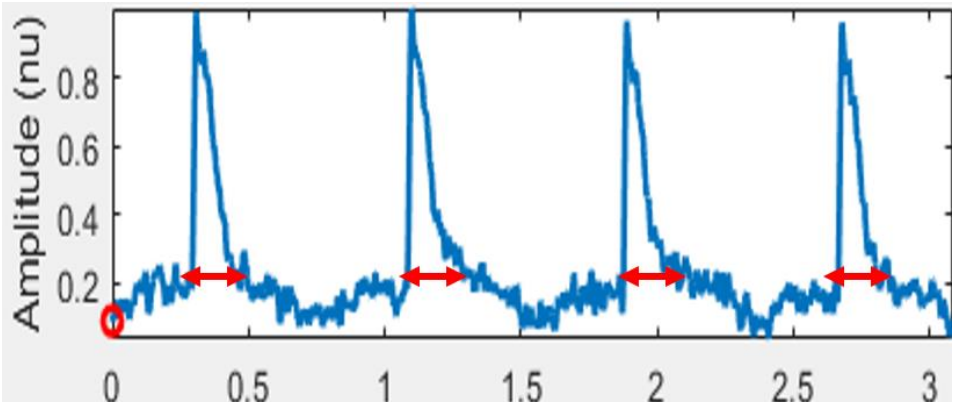

Figure 23. APD calculation from OM data.

For CV calculation and once filtered by selecting two pixels in the Guided User Interface of the specialized software of OM analysis, the program gives in return a value for CV between the two pixels.

The program correlates the two fluorescent signals correspondent to the pixels and with a calibrated distance (spatial information) (that must be performed at the beginning of the process) the CV is calculated with the electric impulse correlation (temporal information).

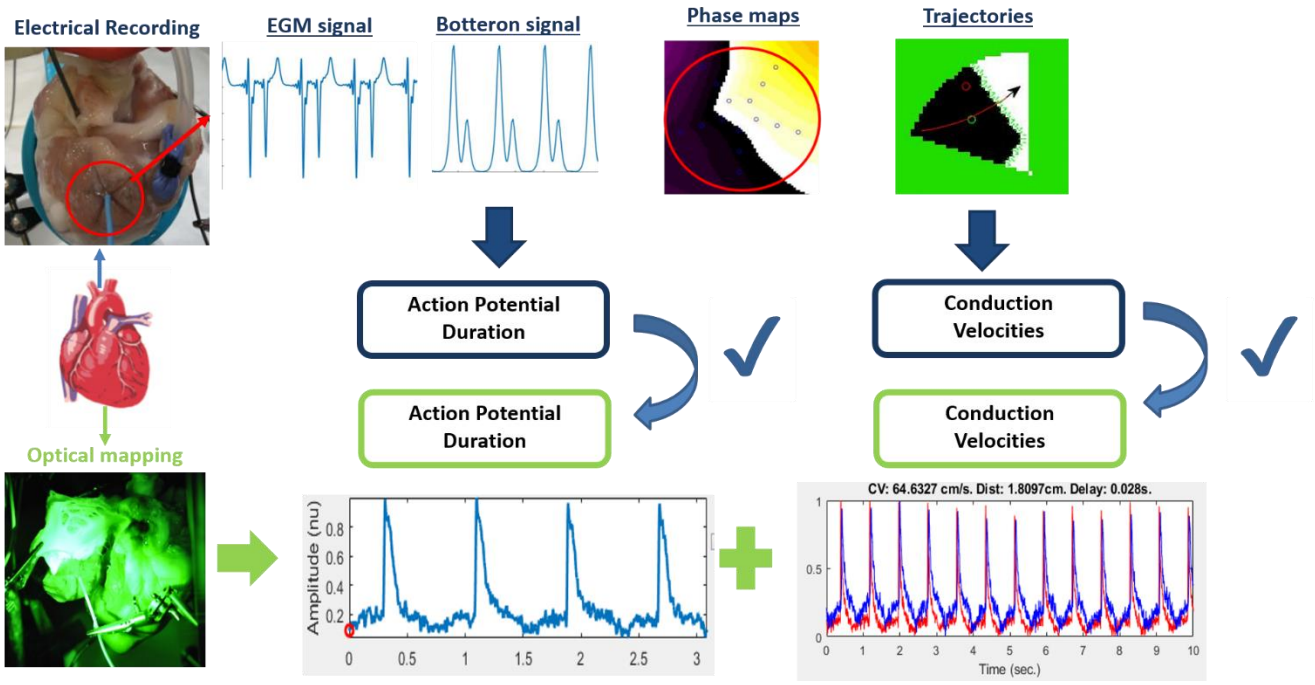

Figure 24. Summary of the results of each step of the software development process compared with the gold standard ones.

- ## Statistical analysis

In order to analyse the correlation between the electrical and optical mapping data two steps were followed:

1. Absolute and relative error calculation were calculated for APD and CV results. Since what is important is to be able to identify changes in APD and CV values, it was set as an acceptable result a difference on 20 ms in APD calculation and 20 cm/s in CV calculation. This interval was selected taking into account that not exact measurements are required for identification of critical areas, just appreciation of relevant differences is needed.
2. T-student statistic test for the acceptable results obtained from the analysis of the absolute errors. Since different recordings were not performed exactly in the same conditions (spacing rates, stimulator catheter location, time elapsed after heart's isolation) data were normalised in pairs (each OM result was set as 1 and electrical value referred to it) to perform the test.

Results were grouped attending to the catheter utilised or the number of electrodes interpolated in order to determine which was the combination that led to the most accurate results.

Results

This bachelor thesis consisted on the development of a software tool able to compute cardiac AF biomarkers such as APD and CV. Even though the main effort and work was devoted to the development of the software, a considerable part of the project itself consisted on the correct execution of the experimental data collection (both the electrical registers and the optical mapping validation ones).

Results presented in this section are the ones corresponding to the recordings of the last experiment as previously mentioned, when the implementation of the experimental process and of the software was completed.

Analysis

Although the objective was to obtain the APD and CV values and validate them with the ones obtained with the optical mapping data, during the process to compute them, phase maps and trajectories (i.e. direction of the impulse at each moment) were also obtained. Even if they were not further analysed more than as an intermediate step in the code process they might be interesting for their use in future studies.

A statistical analysis was performed to analyse the results obtained from the signals recorded from the last experiment. Signals in this last experiment were recorded while pacing at different rates: 800, 500 and 300 ms, and with both the large and the small catheter in the atria of the isolated swine heart.

Analysis: APD and CV

In this section the results from the APD and the CV are presented. In both cases results are analysed on the basis of the catheter utilised and of the 5 or 15 electrode interpolation use in order to determine which option conferred better results.

- Action Potential Duration

The developed software was able to compute APD both with the signals recorded with the small and the large catheter and also from utilizing the outermost 5 electrodes or de 15 available from each catheter highly accurately.

The algorithm estimated the APD accurately for pacing rates higher than 300 ms. The code developed during this project was intended to work at nearby sinus rhythm (frequency is around 1 Hz). Therefore, as frequency recorded from optical mapping when stimulated at 300 ms was around 3.5 Hz and APDs were shortened to the order of 120 ms, it is reasonable that the code was not able to estimate APD under these conditions. Nevertheless, software was able to discern if the signals recorded came from elevated, abnormal pacing rates (characteristic of fibrillation).

In Figure 25 results from the APD computation are presented. As it can be observed, the results obtained from the processing of the EGM signals reproduced the values obtained from the optical mapping data. Not significant differences were observed between the small and large catheter capabilities to sense APDs in the recorded signals according to the T-Student test

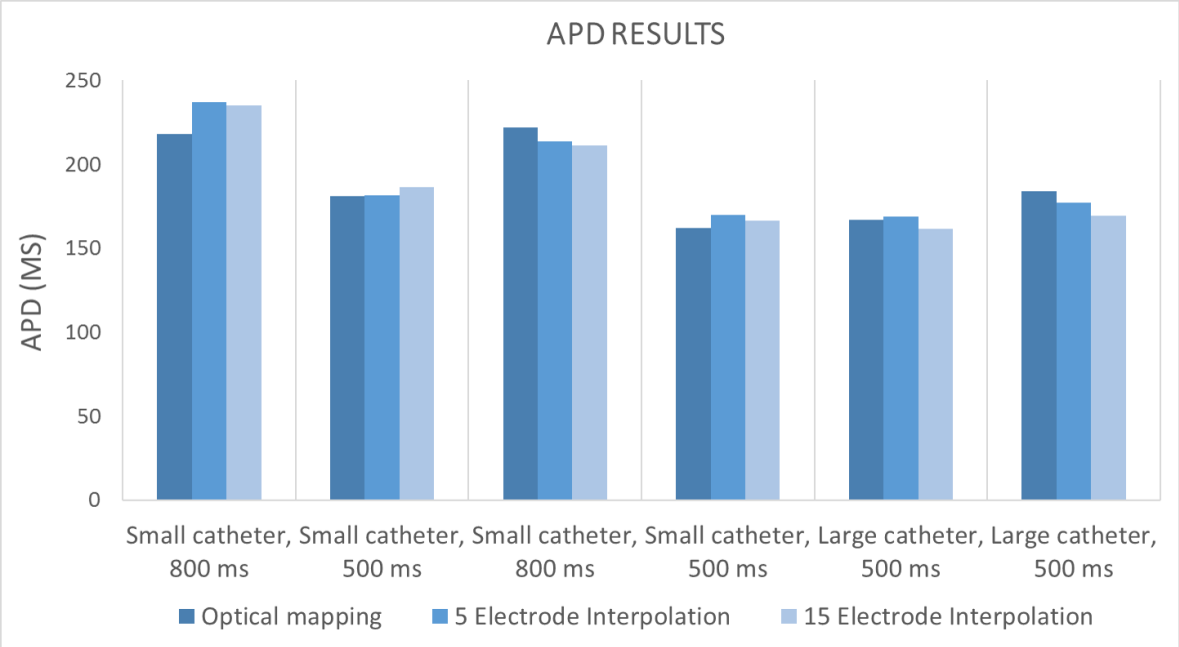

*Figure 25. Results from APD computation and comparison with gold standard data. (*repeated frequency/catheter data was recorded stimulating from different location of the atria)*

In order to ensure the code sensibility and quality of APDs recorded, an analysis including absolute and relative errors and T-student test was performed. 15 electrodes and 5 electrode interpolation were submitted independently to this analysis to test whether there were significant differences between the two interpolations for the APDs computation.

In Table 1 absolute and relative errors from APDs computation are presented. As it can be observed, all absolute errors from APDs obtained are in less than 20 ms (which is between the reasonable range since a difference of 20 ms represent a notable difference to discern two areas with different APD lengths). Relative errors obtained were always minor than 6% what represent highly accurate results.

APD measurements	**5 Electrode Interpolation**		**15 electrode Interpolation**	
	$\overline{E_a}$ *(ms)*	$\overline{E_r}$ *(%)*	$\overline{E_a}$ *(ms)*	$\overline{E_r}$ *(%)*
Small catheter	8.99	4.37%	9.42	4.35%
Large Catheter	4.37	2.43%	10.08	5.63%

Table 1. Results from APD computation: absolute error and relative errors presented.

T-student test results comparing the APDs obtained from 5 electrode interpolation vs. 15 electrode interpolation are showed in Figure 26. Level of confidence was set to α=0.05 and results did not show significant differences when p-value was higher than α. In both cases of APD calculation results did not present statistically relevant differences.

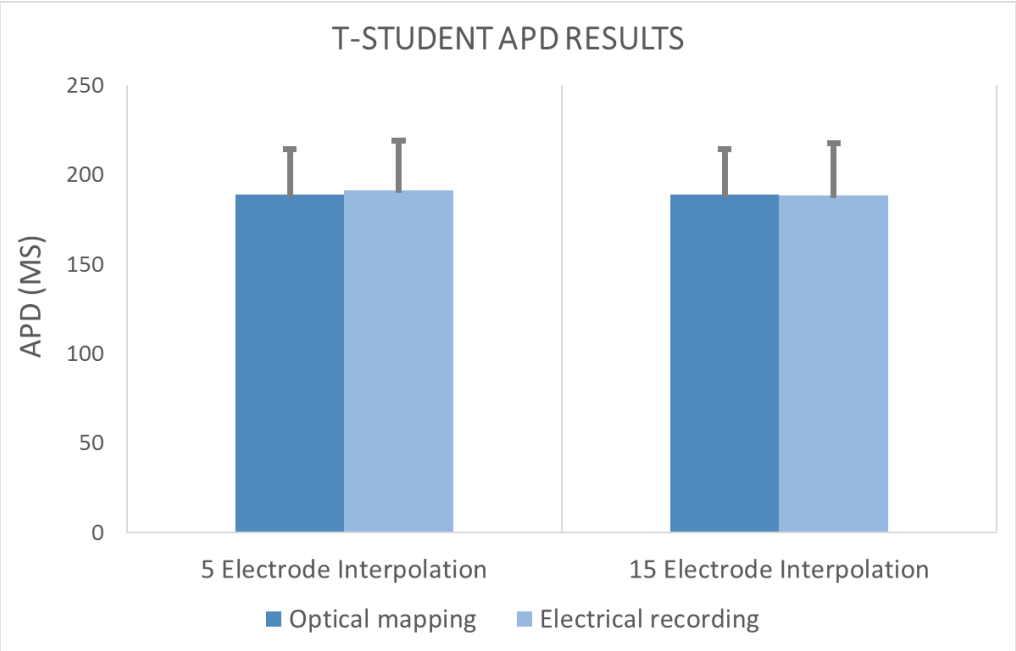

Figure 26. T-student test results from APDs obtained from the 5 electrode interpolation (left) and 15 electrode interpolation (right).

- Conduction Velocity

Conduction velocities results are presented in the following figure [Figure 27].

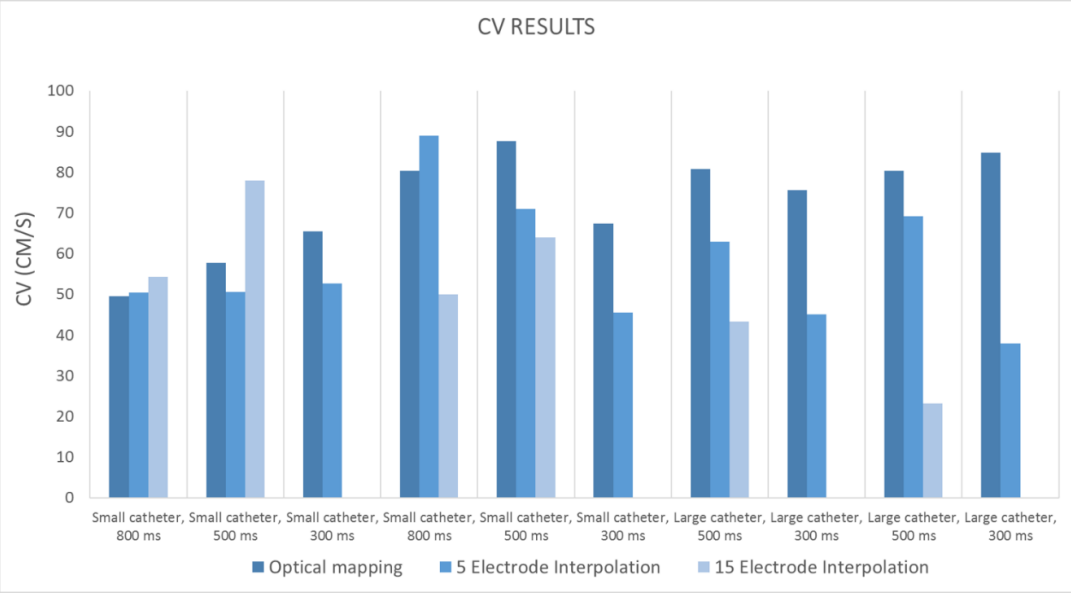

Figure 27. CV global results.

Unlike APD results, the accuracy in CV results showed a higher dependency both on catheter utilised and number of electrode used. Results showed that CV computed were in the acceptable range proposed in this thesis and the results that differed too much came from

the 300 ms signals that, as was previously stated, was set as the limit for nearby sinus rhythms.

Large catheter showed less similitude with optical mapping than the small catheter. Results also differed from 15 electrode interpolation and 5 electrodes interpolation ones showing initially a higher accuracy 5 electrode ones. Further analysis of relative and absolute errors as well as T-student test are presented to confirm the accuracy of the results.

In Table 2 relative and absolute errors attending to catheter type and number of electrodes interpolated are shown. Accepted absolute error was considered to be 20 cm/s so , while all results obtained from the small catheter are in the accepted range while only the 5 electrode interpolation (without the 300 ms signals) from the large catheter is accepted. This difference may be due to the fact that velocities are computed from phase maps and they were better computed from single circles rather than from several point not encoding a clear region. An implementation option would be to compute velocities from the three different rings of five electrodes available in each catheter.

CV MEASUREMENTS	**5 Electrode Interpolation**				**15 Electrode Interpolation**	
	w/ 300 ms		wo/ 300ms		wo/ 300 ms	
	$\overline{E_a}$ *(cm/s)*	$\overline{E_r}$ *(%)*	$\overline{E_a}$ *(cm/s)*	$\overline{E_r}$ *(%)*	$\overline{E_a}$ *(cm/s)*	$\overline{E_r}$ *(%)*
Small Catheter	11.33	15.98%	8.34	11%	19.72	27.33%
Large Catheter	26.56	32.9%	14.45	17.93%	47.11	58.71%

Table 2. CV measurements error results attending to catheter type and number of electrodes interpolated.

A T-student test was performed on the normalized results (excluding 300 ms signals) in order to ensure the results from the small vs. large catheter comparison. Results presented in Figure 28 corroborated that small catheter results correlate better with OM data and, moreover, did not showed significant differences.

To further evaluate the accuracy of the results obtained from the small catheter register another T-student test was performed to analyse the results attending to the number of electrodes used to perform the interpolation [Figure 29].

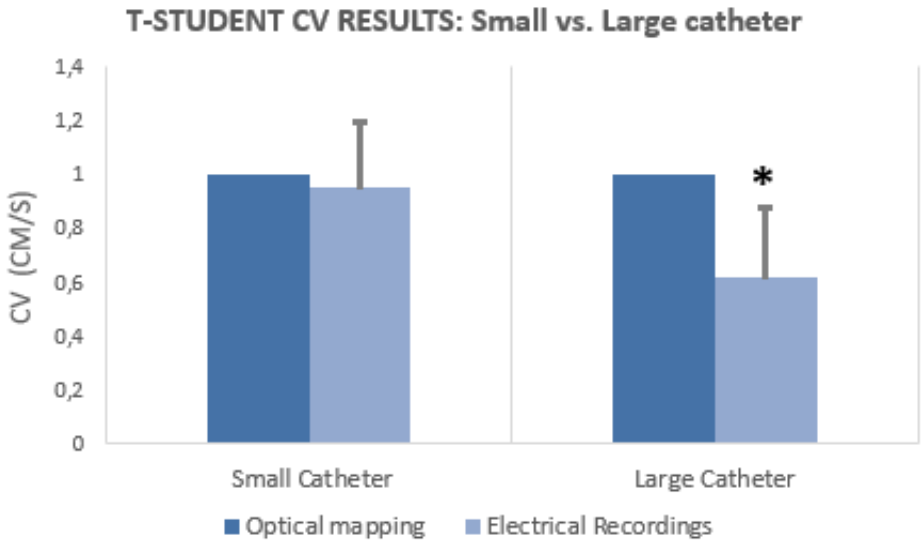

Figure 28. Small vs. Large catheter CV T-student test.

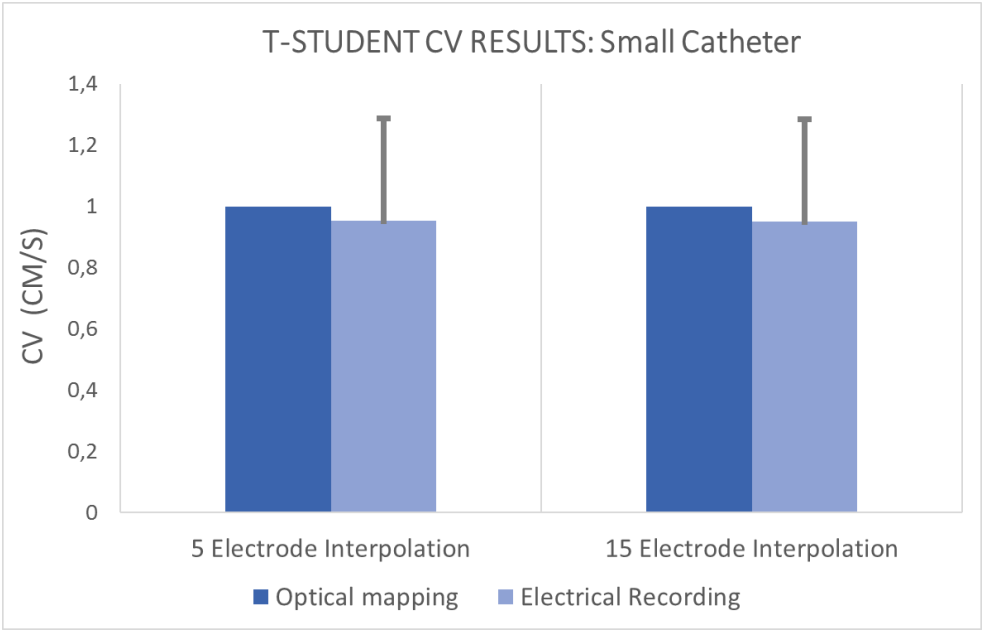

Figure 29. T-student test of CV results obtained from small catheter comparing 15 vs 5 electrodes interpolated.

Although the absolute error obtained from the 5 electrode interpolation was less than the 15 electrode interpolation ones, T-student test results found that differences in both groups of CV values obtained are not statistically significant compared to OM data.

Discussion

This bachelor thesis arises from the need of a validated method for the identification of AF biomarkers. Hypothesis for this project was raised after an intense study of the current studies and existing bibliography regarding AF trigger and maintenance mechanisms and the limitations and problems related with their study and obtaining.

Results obtained for APD computation are considered accurate and no significant differences were observed when utilizing different catheters nor when interpolating signals from all or just one third of the electrodes available. Catheter sensibility to detect both repolarization and depolarization changes in membrane potentials of the heart was proved as well as the code ability to properly analyse the signals in order to obtain APDs either with 5 and 15 electrodes interpolated. Ability of the code to compute APDs with less number of electrodes than the available ones provides an important advantage because it implies that, although failure of some electrode or inability to make proper contact of some of them, results would still be precise.

Regarding CV results, small catheter results both from 15 and 5 electrodes interpolation did not present statically significant differences and were in the variation range set as acceptable for CV. In particular, 5 electrode interpolation gave a higher accuracy: an absolute error of 8.34 vs. 19.72 cm/s (11% and 27.33% relative errors respectively). Large catheter results showed in general statistically significant differences with respect to OM data. Although results from 5 electrode interpolations with large catheter were inside the range of acceptable CV variations, small catheter results were better. Therefore, it can be concluded that the best CV results were achieved with the small catheter signals interpolated from 5 electrodes.

Differences between large and small catheter may be due to the size of the catheter since recording in the atria, small catheter is able to make a better fit (2.5 vs. 3.5 cm). Atria surface is reduced, and in order to make the proper measurements and fit the catheter, sometimes a balloon had to be introduced inside the atria to inflate it and expand its surface. However, even with the large catheter, an absolute error of 14.45 cm/s (acceptable to determine slow CV regions, and to determine a target ablation area) was obtained.

In conclusion, CV and APDs results presented the accuracy desired to be able to determine the areas prone to harbor the trigger AF and thus, set a target for radiofrequency ablation. Application to clinical area of the results obtained from this thesis may result in more

specific treatments. Currently, ablation therapy is performed by applying a general protocol that, in case to be able to determine the critical regions for ablation, may be designed specifically for each patient improving the results of the intervention and probably reducing the number of interventions needed, making it a long-term treatment.

This thesis arises from the need of a validated method for the interpretation of intracardiac EGM signals. Considerable approaches have been made in order to analyze them and obtain useful information about cardiac function and its assessment. Several approaches intended to calculate CV from local activation time, based on cycle length and time-domain analysis [55]. Problem is that results are recording-mode dependent, unipolar or bipolar recording will greatly influence the obtained signals. Approaches intended to develop solutions for those interferences are also considered [43]. However, these approaches have been demonstrated to not correlate well when validated with OM data [17].

New studies suggested the use of phase as a robust method for intracardiac EGM analysis [45]. Kuklik et al. [46] proposed a method based in Hilbert Transform to obtain phase from unipolar electrograms that, although not developed for fibrillation rhythms was utilized as a first step for this thesis. Phase calculation was the basis for several studies that served to deepen in EGM interpretation as for example the one proposed for Roney et. Al [48] that studied the influence of spatial resolution requirements on mathematical simulations of EGM as well as propose the method for phase interpolation used in this thesis. However, none of these methods proposed a validated process to obtain biomarkers related to AF as this thesis is intended for.

Software code developed in this project implemented a combination of several proposed intracardiac processing methods such as the previous ones mentioned as well as the signal processing algorithm proposed by Botteron [65]. This thesis was validated with optical mapping data, which is a far validated and reliable technique that, added to the fact that validation of the results was performed with data obtained *ex vivo,* results in a major implementation towards clinical application of the code, which is the major end of this technology.

Limitations

This section aims to be critical with the development of the project since it is really important to understand the backdrops of the study and be aware of the limitations that it dealt with (some of which are common to related studies as well) in order to be able to continue developing it:

- As it was shown in the previous section, the code was whether not able to obtain such biomarkers values from the 300 ms data or when computed showed a bad correlation with gold standard data (even the code works properly for its aimed function: sinus rhythm nearby 1 Hz).

- Results and precision of the software, beyond the accuracy of the method itself, are also conditioned to the sensibility that intracardiac catheters possess themselves to measure the electrical changes.

- One of the assumptions made in this project had to do with the geometry and position of the catheter during registers. Software code was developed supposing that the catheter was fully extended, the arms were equally distributed and fitted onto a flat surface. During clinical electrophysiological interventions, such as an ablation for example, a sophisticated localization software informs about the precise location of each of the catheters within the body, and thus within the heart. However, in the experiments performed for this thesis it was no possible to take into account these considerations. Variations introduced by making these assumptions, although thought to be small, can improve the results making them even more accurate.

- Contact of the catheter's electrodes with the heart's surface may greatly influence the results. Although in the experiments a lot of effort was put in order to ensure the best possible fitting between the catheter and the heart's tissue, some electrodes might not be making the proper contact. As well in a real clinical electrophysiological intervention this possible event must be taken into account.

- Availability of signals was limited due to technical problems related with the electronic equipment of the Langendorff set-up and validation of results was performed with a reduced number of samples.

Future work

The developed software in this bachelor should be further validated with an increased number of sample signals recorded to totally ensure its accuracy and be able to determine exactly its sensibility depending on the recording conditions.

Next steps should be intended to continue the hypothesis raised during this project and incorporate this technology to the tools already utilized to comprehend the mechanism underlying and perpetuating AF. Also, software code may be implemented in order to be able to compute APDs and CV in real-time guiding it to a feasible clinical application. Also, to achieve the goal of the clinical application of the code proposed in this thesis, further analysis and validation must be performed in *in-vivo* situations and to determine the effectiveness of ablation of the regions detected by this code.

Although the software developed during this project was not intended to work during the arrhythmia itself, natural next step should be the implementation of the code in order to improve its performance during close-to-fibrillation rhythms.

Not only code-related implementation but also regarding the catheters. Another configurations, geometries and electrode densities could be tested in order to ensure the optimal performance. As a hypothesis, including a central electrode could improve the sensing catheter properties in general (in the case of pentarray-like catheters) and in particular, its inclusion, creating a more equally distributed network of electrodes, could improve the results of the code (as the phase interpolation results would be enhanced).

Although it was out of the scope of this project, during the analysis of the optical mapping data it can be appreciated that conduction velocities within the vicinity of the catheter location were somehow altered. Even this fact did not alter the results of the validation process of the project (although the CV differed from the values of the rest of the heart they were the same CV values the ones measured electrically than with optical mapping) it is thought to be interesting to investigate and assess the effect of the catheters on the effect of the catheters on the electrophysiological properties of the organ.

Expenditure, Socioeconomic Impact and Legal Framework

Expenditure

Expenditure section will be detailed in three different parts: human resources, software development and data collection experiments.

Human Resources	**Invested Hours**	**Cost (€/hour)**	**Total (€)**
Biomedical Engineer	400	20	8000
Project Collaborator	100	35	3500
Project Coordinator	80	35	2800
		Total	**14300 €**

Table 3. Human Resources Expenditure.

Software Resources	**Units**	**Price/Unit (€)**	**Amortisation period**	**Total (€)**
MATLAB®2017b	1	2000 (perpetual)	5 years	400
Computer	1	800	5 years	160
			Total	**560 €**

Table 4. Software Resources Expenditure.

Data Collection Experiments*	**Units**	**Price/Unit (€)**	**Amortisation period**	**Number of experiments**	**Total (€)**
Langendorff System set-up + Optical mapping set-up	1	57,000	200 experiments	7	1,995
Polygraph 64 channels	1	12,000	200 experiments	7	200
*Swine heart isolation***	1	590	-	7	4130
				Total	**6325 €**

Table 5. Data Collection Experiments Expenditure.

**All expenses were shared with another project developed in the laboratory.*

*** Following the cost tables of animals and procedures of the Fundación para la Investigación Biomédica del Hospital Gregorio Marañón where the procedures were carried out, the estimated cost per animal of 3 months with an estimated weight of 45kg and for the cost of renting the surgical room and support from the team of veterinarians and anesthetists. It is included in the cost the necessary fungible material (throw, fluorophores, electrodes, etc.).*

Total expenditure of the project was **21185 €.**

Socioeconomic Impact

Atrial Fibrillation is the most common arrhythmia and it is expected that by 2025, 2% of the population would be affected by AF [66]. More than a health issue, this adds up to a huge economic impact on the healthcare systems [67].

Therefore, ideas to treat AF point in the direction of prevention and more specific treatments. Currently, ablation procedures are the most effective treatment for AF but lack of identification of focal sources of initiation of the arrhythmia make these treatments not long-term efficient and in need for several interventions.

Application of this thesis to basic research may help understanding the mechanisms underlying the initiation and maintenance of AF and thus, helping in prevention of arrhythmias and improving the current treatments.

APD and CV computation from intracardiac EGM signals allows for the identification of the regions prone to harbour the trigger of AF. The code was implemented for sinus rhythm since patients with paroxysmal AF ablation (which are the ones indicated for ablation therapy) are normally in sinus rhythm so by locating the regions with shortened APDs and reduced CV, target for ablation would be identified. Application to clinical practice would have an important economic impact if it allows for a real target ablation procedure: number of interventions will decrease, number of hospitalizations will be reduced as well and both human quality of life and economic impact of AF in healthcare systems will be improved.

Legal Framework

As it was previously mentioned experiments with swine hearts realized for this thesis were approved by the Ethics Committee on Animal Experimentation (EAEC) of the Hospital Universitario Gregorio Marañón.

The EAEC of Gregorio Marañón is in charge of these issues, to monitor the implementation of animals in the laboratory as established in current legislation. Also, it should oversee the project in order to check if everything is done in relation to the rights and welfare of animals, persons and environment. Article 38 of RD 53/2013.

"Real Decreto 53/2013, de 1 de febrero, por el que se establecen las normas básicas aplicables para la protección de los animales utilizados en experimentación y otros fines científicos, incluyendo la docencia."

The EAEC has the designation of Organ enabled (OH) by the competent body in the Community of Madrid. The main goal of this entity is to evaluate if the projects are scientifically or educationally justified; if the final purpose justified the use of animals and its farewell.

The EAEC will evaluate the projects proposed in the Unit of Medicine and Experimental Surgery of the Gregorio Marañón Hospital (UMCE) and the OH will evaluate the projects proposed to be carried out in the Gregorio Marañón Hospital Health Research Institute (liSGM).

All staff that has been involved in the swine heart extraction/been in contact with the animal were correctly trained according to the RD 53/2013 and the Order ECC 566/2015.

"Orden ECC/566/2015, de 20 de marzo, por la que se establecen los requisitos de capacitación que debe cumplir el personal que maneje animales utilizados, criados o suministrados con fines de experimentación y otros fines científicos, incluyendo la docencia."

Conclusions

This bachelor thesis aimed to develop a method for the computation of APDs and CVs from electrical recordings obtained from intracardiac catheters and to validate it through the values obtained from simultaneous OM data. After the analysis of the results it can be stated that:

- A software code able to easily and automatically compute APDs and CV from intracardiac EGM signals was developed.

- Results computed by the software developed from EGM signals registered in a swine isolated heart were validated and demonstrated to be accurate enough to considerate this method as valid.

    - Specifically, it was demonstrated that with a small catheter (2.5 cm radius) better results were obtained.
    - Number of electrodes interpolated was not relevant for APDs obtaining while regarding CV computation with 5 electrodes instead of 15 electrodes interpolated showed more accurate results.

In conclusion, a software code for computing APDs and CV from intracardiac EGM signals was developed and validated using OM data. Computing of APDs and CV from intracardiac EGM can be used to identify which areas of cardiac tissue are prone to harbour the trigger and beginning of AF. Identification of such areas is clinically relevant because it can be used to obtain the ablation target in patients with paroxysmal AF.

Bibliography

[1] "Global Health Estimates 2015: Disease burden by Cause, Age, Sex, by Country and by Region, 2000-2015. Geneva, World Health Organization," 2016.

[2] E. Wilkins *et al.*, "European Cardiovascular Disease Statistics 2017. European Heart Network, Brussels," 2017.

[3] E. J. Benjamin *et al.*, "Heart Disease and Stroke Statistics—2017 Update: A Report from the American Heart Association," *Circulation*, vol. 135, no. 10, pp. e146–e603, Mar. 2017.

[4] K. S. McDowell, S. Zahid, F. Vadakkumpadan, J. Blauer, R. S. MacLeod, and N. A. Trayanova, "Virtual Electrophysiological Study of Atrial Fibrillation in Fibrotic Remodeling," *PLOS ONE*, vol. 10, no. 2, p. e0117110, Feb. 2015.

[5] Developed with the special contribution of the European Heart Rhythm Association (EHRA) *et al.*, "Guidelines for the management of atrial fibrillation: The Task Force for the Management of Atrial Fibrillation of the European Society of Cardiology (ESC)," *European Heart Journal*, vol. 31, no. 19, pp. 2369–2429, Oct. 2010.

[6] J. Pérez-Villacastín, N. Pérez Castellano, and J. Moreno Planas, "Epidemiología de la fibrilación auricular en España en los últimos 20 años," *Rev Esp Cardiol*, vol. 66, no. 07, pp. 561–565, Jul. 2013.

[7] A. S. Go *et al.*, "Prevalence of Diagnosed Atrial Fibrillation in Adults: National Implications for Rhythm Management and Stroke Prevention: the Anticoagulation and Risk Factors In Atrial Fibrillation (ATRIA) Study," *JAMA: the journal of the American Medical Association*, vol. 285, pp. 2370–5, May 2001.

[8] S. Stewart, C. L. Hart, D. J. Hole, and J. J. V. McMurray, "A population-based study of the long-term risks associated with atrial fibrillation: 20-year follow-up of the Renfrew/Paisley study," *Am. J. Med.*, vol. 113, no. 5, pp. 359–364, Oct. 2002.

[9] J. Jalife, "Déjà vu in the theories of atrial fibrillation dynamics," *Cardiovasc. Res.*, vol. 89, no. 4, pp. 766–775, Mar. 2011.

[10] M. Yamazaki and J. Jalife, "Pathophysiology of atrial fibrillation: From initiation to maintenance," *Journal of Arrhythmia*, vol. 28, no. 3, pp. 129–139, Jun. 2012.

[11] Y. Zheng, Y. Xia, J. Carlson, O. Kongstad, and S. Yuan, “Atrial average conduction velocity in patients with and without paroxysmal atrial fibrillation,” *Clin Physiol Funct Imaging*, vol. 37, no. 6, pp. 596–601, Nov. 2017.

[12] G. G. Lalani, A. Schricker, M. Gibson, A. Rostamian, D. E. Krummen, and S. M. Narayan, “Atrial Conduction Slows Immediately Before the Onset of Human Atrial Fibrillation,” *J Am Coll Cardiol*, vol. 59, no. 6, pp. 595–606, Feb. 2012.

[13] D. E. Krummen *et al.*, “Mechanisms of Human Atrial Fibrillation Initiation: Clinical and Computational Studies of Repolarization Restitution and Activation Latency,” *Circulation: Arrhythmia and Electrophysiology*, vol. 5, no. 6, pp. 1149–1159, Dec. 2012.

[14] M. R. Franz, S. M. Jamal, and S. M. Narayan, “The role of action potential alternans in the initiation of atrial fibrillation in humans: a review and future directions,” *Europace*, vol. 14, no. Suppl 5, p. v58, Nov. 2012.

[15] L. M. Ptaszek, B. Moon, G. Rozen, S. Mahapatra, and M. Mansour, “Novel automated point collection software facilitates rapid, high-density electroanatomical mapping with multiple catheter types,” *J. Cardiovasc. Electrophysiol.*, vol. 29, no. 1, pp. 186–195, Jan. 2018.

[16] E. Anter, C. M. Tschabrunn, A. E. Buxton, and M. E. Josephson, “High-Resolution Mapping of Post-Infarction Reentrant Ventricular Tachycardia: Electrophysiological Characterization of the Circuit,” *Circulation*, vol. 134, no. 4, pp. 314–327, Jul. 2016.

[17] O. Berenfeld *et al.*, “Time- and frequency-domain analyses of atrial fibrillation activation rate: the optical mapping reference,” *Heart Rhythm*, vol. 8, no. 11, pp. 1758–1765, Nov. 2011.

[18] Jaakko Malmivuo and R. Plonsey, *BioelectromagnetismPrinciples and Applications of Bioelectric and Biomagnetic Fields*. Oxford University Press, 1995.

[19] Gerard J. Tortora and Bryan Derrickson, “Chapter 10: The Cardiovascular System: The Heart,” in *Principles of Anatomy and Physiology*, Twelfth., Wiley.

[20] Moini, J, “Chapter 5: Anatomy and Physiology of the Cardiovascular System,” in *Phlebotomy: Principles and Practice*, Jones & Bartlett Publishers, 2013.

[21] “ECGpedia.” [Online]. Available: https://en.ecgpedia.org/index.php?title=Main_Page.

[22] “Categories of Arrhythmias,” *Texas Heart Institute*.

[23] “Heart and Stroke Association Statistics.”

[24] José Jalife, Mario Delmar, Justus Anumonwo, Omar Berenfeld, and Jérôme Kalifa, "Basic Mechanisms of Cardiac Arrhythmias," in *Basic Cardiac Electrophysiology for the Clinician*, Wiley-Blackwell, 2009, pp. 152–190.

[25] S. V. Pandit *et al.*, "Ionic Determinants of Functional Reentry in a 2-D Model of Human Atrial Cells During Simulated Chronic Atrial Fibrillation," *Biophys J*, vol. 88, no. 6, pp. 3806–3821, Jun. 2005.

[26] J. Hall and A. Gyuton, *Textbook of Medical Physiology*, 11th ed. Saunders, 2005.

[27] S. Lévy, "Classification system of atrial fibrillation," *Current Opinion in Cardiology*, vol. 15, no. 1, pp. 54–57, Jan. 2000.

[28] M. S. Guillem, A. M. Climent, M. Rodrigo, F. Fernández-Avilés, F. Atienza, and O. Berenfeld, "Presence and stability of rotors in atrial fibrillation: evidence and therapeutic implications," *Cardiovasc Res*, vol. 109, no. 4, pp. 480–492, Apr. 2016.

[29] A. M. Climent, M. S. Guillem, F. Atienza, and F. Fernández-Avilés, "Electrophysiological characteristics of permanent atrial fibrillation: insights from research models of cardiac remodeling," *Expert Rev Cardiovasc Ther*, vol. 13, no. 1, pp. 1–3, Jan. 2015.

[30] S. Nattel and M. Harada, "Atrial Remodeling and Atrial Fibrillation: Recent Advances and Translational Perspectives," *Journal of the American College of Cardiology*, vol. 63, no. 22, pp. 2335–2345, Jun. 2014.

[31] B. Burstein and S. Nattel, "Atrial fibrosis: mechanisms and clinical relevance in atrial fibrillation," *J. Am. Coll. Cardiol.*, vol. 51, no. 8, pp. 802–809, Feb. 2008.

[32] T. Igarashi *et al.*, "Connexin gene transfer preserves conduction velocity and prevents atrial fibrillation," *Circulation*, vol. 125, no. 2, pp. 216–225, Jan. 2012.

[33] J. Xu, J. G. Y. Luc, and K. Phan, "Atrial fibrillation: review of current treatment strategies," *J Thorac Dis*, vol. 8, no. 9, pp. E886–E900, Sep. 2016.

[34] "Guidelines for the management of atrial fibrillation | European Heart Journal | Oxford Academic." [Online]. Available: https://academic.oup.com/eurheartj/article/31/19/2369/442190. [Accessed: 03-Jun-2018].

[35] "Classifications of Antiarrhythmic Agents: Classifications of Antiarrhythmic Agents," Jan. 2017.

[36] "Cardioversion in atrial fibrillation described." [Online]. Available: https://www.escardio.org/Journals/E-Journal-of-Cardiology-Practice/Volume-11/Cardioversion-in-Atrial-Fibrillation-Described.

[37] “Cardioversión - Mayo Clinic.” [Online]. Available: https://www.mayoclinic.org/es-es/tests-procedures/cardioversion/about/pac-20385123. [Accessed: 03-Jun-2018].

[38] R. Cappato *et al.*, “Updated worldwide survey on the methods, efficacy, and safety of catheter ablation for human atrial fibrillation,” *Circ Arrhythm Electrophysiol*, vol. 3, no. 1, pp. 32–38, Feb. 2010.

[39] H. Calkins *et al.*, “2012 HRS/EHRA/ECAS Expert Consensus Statement on Catheter and Surgical Ablation of Atrial Fibrillation: recommendations for patient selection, procedural techniques, patient management and follow-up, definitions, endpoints, and research trial design,” *Europace*, vol. 14, no. 4, pp. 528–606, Apr. 2012.

[40] Y. Takahashi, S. Yamashita, M. Suzuki, K. Yabe, and K. Hirao, “Efficacy of catheter ablation of focal sources in persistent atrial fibrillation,” *J. Cardiovasc. Electrophysiol.*, vol. 29, no. 4, pp. 559–565, Apr. 2018.

[41] W. S. Tzou *et al.*, “Long-term outcome after successful catheter ablation of atrial fibrillation,” *Circ Arrhythm Electrophysiol*, vol. 3, no. 3, pp. 237–242, Jun. 2010.

[42] “Atrial Fibrillation Ablation,” *Arizona Heart Rhythm Center*.

[43] S. Massé *et al.*, “Resolving Myocardial Activation With Novel Omnipolar Electrograms,” *Circulation: Arrhythmia and Electrophysiology*, vol. 9, no. 7, p. e004107, Jul. 2016.

[44] C. D. Cantwell, C. H. Roney, F. S. Ng, J. H. Siggers, S. J. Sherwin, and N. S. Peters, “Techniques for automated local activation time annotation and conduction velocity estimation in cardiac mapping,” *Comput Biol Med*, vol. 65, pp. 229–242, Oct. 2015.

[45] K. Umapathy *et al.*, “Phase Mapping of Cardiac Fibrillation,” *Circulation: Arrhythmia and Electrophysiology*, vol. 3, no. 1, pp. 105–114, Feb. 2010.

[46] P. Kuklik *et al.*, “Reconstruction of Instantaneous Phase of Unipolar Atrial Contact Electrogram Using a Concept of Sinusoidal Recomposition and Hilbert Transform,” *IEEE transactions on bio-medical engineering*, vol. 62, Aug. 2014.

[47] M. P. Nash *et al.*, “Evidence for Multiple Mechanisms in Human Ventricular Fibrillation,” *Circulation*, vol. 114, no. 6, pp. 536–542, Aug. 2006.

[48] C. H. Roney *et al.*, “Spatial Resolution Requirements for Accurate Identification of Drivers of Atrial Fibrillation,” *Circ Arrhythm Electrophysiol*, vol. 10, no. 5, p. e004899, May 2017.

[49] M. Rodrigo, A. M. Climent, S. M. Guillem, F. Atienza, and O. Berenfeld, “Catéter y método para la detección de actividad eléctrica en un órgano,” 2 529 702.

[50] J. H. King, C. L.-H. Huang, and J. A. Fraser, “Determinants of myocardial conduction velocity: implications for arrhythmogenesis,” *Front Physiol*, vol. 4, Jun. 2013.

[51] D. E. Krummen *et al.*, “Mechanisms for Human Atrial Fibrillation Initiation: Clinical and Computational Studies of Repolarization Restitution and Activation Latency,” *Circ Arrhythm Electrophysiol*, vol. 5, no. 6, Dec. 2012.

[52] R. Liao, B. K. Podesser, and C. C. Lim, “The continuing evolution of the Langendorff and ejecting murine heart: new advances in cardiac phenotyping,” *American Journal of Physiology-Heart and Circulatory Physiology*, vol. 303, no. 2, pp. H156–H167, Jul. 2012.

[53] R. M. Bell, M. M. Mocanu, and D. M. Yellon, “Retrograde heart perfusion: The Langendorff technique of isolated heart perfusion,” *Journal of Molecular and Cellular Cardiology*, vol. 50, no. 6, pp. 940–950, Jun. 2011.

[54] W. E. Louch, K. A. Sheehan, and B. M. Wolska, “Methods in cardiomyocyte isolation, culture, and gene transfer,” *Journal of Molecular and Cellular Cardiology*, vol. 51, no. 3, pp. 288–298, Sep. 2011.

[55] J. I. Laughner, F. S. Ng, M. S. Sulkin, R. M. Arthur, and I. R. Efimov, “Processing and analysis of cardiac optical mapping data obtained with potentiometric dyes,” *Am J Physiol Heart Circ Physiol*, vol. 303, no. 7, pp. H753–H765, Oct. 2012.

[56] M. Attin and W. T. Clusin, “Basic Concepts of Optical Mapping Techniques in Cardiac Electrophysiology,” *Biol Res Nurs*, vol. 11, no. 2, pp. 195–207, Oct. 2009.

[57] T. J. Herron, P. Lee, and J. Jalife, “Optical Imaging of Voltage and Calcium in Cardiac Cells & Tissues,” *Circ Res*, vol. 110, no. 4, pp. 609–623, Feb. 2012.

[58] K. R. Laurita, S. D. Girouard, and D. S. Rosenbaum, “Modulation of Ventricular Repolarization by a Premature Stimulus: Role of Epicardial Dispersion of Repolarization Kinetics Demonstrated by Optical Mapping of the Intact Guinea Pig Heart,” *Circulation Research*, vol. 79, no. 3, pp. 493–503, Sep. 1996.

[59] M. Morad and G. Salama, “Optical probes of membrane potential in heart muscle,” *J. Physiol. (Lond.)*, vol. 292, pp. 267–295, Jul. 1979.

[60] E. Fluhler, V. G. Burnham, and L. M. Loew, “Spectra, membrane binding, and potentiometric responses of new charge shift probes,” *Biochemistry*, vol. 24, no. 21, pp. 5749–5755, Oct. 1985.

[61] E. Entcheva and H. Bien, “Macroscopic optical mapping of excitation in cardiac cell networks with ultra-high spatiotemporal resolution,” *Prog. Biophys. Mol. Biol.*, vol. 92, no. 2, pp. 232–257, Oct. 2006.

[62] J. W. Lichtman and J.-A. Conchello, “Fluorescence microscopy,” *Nat. Methods*, vol. 2, no. 12, pp. 910–919, Dec. 2005.

[63] S. K. Madan, B. Bhaumik, and J. M. Vasi, “Experimental observation of avalanche multiplication in charge-coupled devices,” *IEEE Transactions on Electron Devices*, vol. 30, no. 6, pp. 694–699, Jun. 1983.

[64] E. Garreta *et al.*, “Myocardial commitment from human pluripotent stem cells: Rapid production of human heart grafts,” *Biomaterials*, vol. 98, pp. 64–78, 2016.

[65] W. Li, C. Yang, Y. Wang, D. Wang, Y. Chen, and Z. Wu, “Several insights into the preprocessing of electrograms in atrial fibrillation for dominant frequency analysis,” *Biomed Eng Online*, vol. 15, p. 38, Apr. 2016.

[66] A. J. Camm, “Atrial Fibrillation and Risk,” *Clinical Cardiology*, vol. 35, no. S1, pp. S1–S2.

[67] R. J. A. Till and M. R. Cowie, “Atrial Fibrillation In Heart Failure: New Directions In Diagnosis, Risk Assessment And Risk Reduction,” *J Atr Fibrillation*, vol. 6, no. 6, Apr. 2014.